THE USE OF IONIZING RADIATION FOR THE HYDROLYSIS OF PECTINS AND THE EFFECT ON THE PROPERTIES OF THE EXTRACTED PECTINS

By

ABDEL-HAMID A. KASSEM

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INTRODUCTION

The goal of pectin manufacture is to obtain a product representing the highest jelly grade and yield economically from the raw material. The white spongy albedo of citrus fruits is eminently suitable as a raw product for pectin manufacture. Most of the pectin produced in the United States is now made from citrus peel (Agriculture Handbook No. 98, 1962). The production of frozen concentrated citrus juices has now reached exceedingly high levels, and the citrus residue left as a by-product is enormous. The classical methods of extraction and separation of pectins have been described by Wilson (1925), Myers et al. (1929), and Owens et al. (1949). The principle problem of these methods is the proper selection of the pH, time and temperature of the extraction to enhance dissolution of the pectin (Kertesz, 1951).

Radiation preservation of foods has become increasingly important, and extensive research has been conducted for the use of gamma radiation for the extension of shelf life of citrus fruits. Direct observations on the alteration and destruction of the cellular pectic constituents caused by radiation have been made by means of light and electron microscopy (Roberts et al., 1955). However, only a few investigations were made relating the characterization of pectins extracted from fruit

exposed to varying doses of radiation. In one of the few investigations on the effect of irradiation on properties of pectins, Rouse et al. (1968) showed an increase in yield accompanied by a decrease in grade. No optimization study regarding jelly units was ever conducted.

This study was designed to investigate the effect of gamma radiation, at varying dose levels, on the characteristics of citrus pectin from citrus residue and the possibility of using radiation hydrolysis instead of acid hydrolysis for the commercial production of pectin.

LITERATURE REVIEW

Nomenclature

The history of pectin chemistry is about 130 years old, and the nomenclature of the pectic substances has been very confusing and contradictory. The first successful attempt to bring order and an accepted nomenclature was made in 1926 by a committee of the American Chemical Society (Brinton et al., 1927). The following is a revision of this nomenclature which was brought about by the Society in 1943 and adopted as official in 1944 (Baker et al., 1944).

> Pectic substances: This term is a group designation for those complex colloidal carbohydrate derivatives which occur in, or are prepared from, plants and contain a large proportion of anhydrogalacturonic acid units which are thought to exist in a chain-like combination. The carboxyl groups of polygalacturonic acids may be partly esterified by methyl groups and partly or completly neutralized by one or more bases.
> Protopectin: This term is applied to the water-insoluble parent pectic substance which occurs in plants and which, upon restricted hydrolysis, yields pectinic acids.
> Pectinic acids: This term is used for colloidal polygalacturonic acids containing more than a negligible proportion of methyl ester groups. Pectinic acids under suitable conditions, are capable of forming gels (jellies) with sugar and acid, or if suitably low in methoxyl content, with certain metallic ions. The salts of

pectinic acids are either normal or acid pectinates.
Pectin: This term designates those water-soluble pectinic acids of varying methyl ester content and degree of neutralization which are capable of forming gels with sugar and acid under suitable conditions.
Pectic acid: This term is applied to pectic substances mostly composed of colloidal polygalacturonic acids and essentially free from methyl ester groups. The salts of pectic acid are either normal or acid pectates.

Extraction

The development of the pectin industry in the United States prior to 1928 was reviewed by Rooker (1928). At that time much of the pectin was put out as a pectin syrup, and its manufacture consisted of the following steps; (1) Grinding and pressing the apples (apples were the main source of pectin), (2) Drying the resulting pomace, (3) Leaching the pomace with cold water, (4) Extracting the pectin, (5) Pressing the treated mass, (6) Partly clarifying the pectin juice by means of centrifuges or settling tanks, (7) Treating the juice with proper enzymes to remove starches and proteins, (8) Treating the juice with decolorizing carbons, (9) Filtration of the juice to the desired degree of brilliancy, (10) Concentration of the filtrate in vacuum, (11) Adjusting the finished syrup as to acid content and jelly strength, (12) Bottling or canning the adjusted syrup, (13) Pasteurizing the syrup in the containers. In the manufacture of powdered pectin the filtrate was spray dried or the concentrated syrup was drum dried.

The extraction of the pectin was done using acidified hot water. The times and temperatures recommended were from 30 to 80 minutes at 190 to 212° F. For acidification there are many references in the literature relative to the use of tartaric acid for this purpose, advising the use of a 0.1% solution (Mehlitz, 1925). Rooker (1928) prefers to use a 0.2% solution of lactic acid.

The extraction of pectins in manufacturing has gone a long way since then, to the point where today the use of gamma radiation is being considered as a possible method for hydrolysis (Rouse, 1968 a). Wilson (1925) was one of the first researchers to consider the use of a cheap mineral acid for the extraction of pectins. He found that the use of sulphurous acid has many advantages such as the possibility of removing it by aeration and its bleaching effect. Its main disadvantage was the difficulty in controlling the pH of the solution.

An important development in the extraction of pectin was the use of polyphosphates. Norris et al. (1925) were the first to elaborate on this topic after which Nanji et al. (1927) obtained a patent on the use of salts which form insoluble compounds with calcium and magnesium to facilitate the extraction of pectin.

Myers et al. (1929) compared the properties of pectins extracted by boiling for 20 minutes while varying the pH by the addition of tartaric acid, or hydrochloric acid. They found that regardless of the amount and nature of the acid used, a pectin with maximum jelly units was obtained when it was extracted

at a hydrogen ion concentration of pH 2.15. They also developed a modified method of extraction using hydrochloric acid which has become one of the most widely used (Myers et al., 1932).

A more recent method was that of Myers et al. (1943) who used ion-exchange agents for pectin extraction. In this process the pectin source was ground and washed to remove electrolytes. It was then mixed with Zeo-Karb H (a sulphonated coal) and heated at 90° C for one hour. The exchange agent removes the calcium and magnesium from the extract, lowers the pH, and liberated the pectin. The source and exchange agent are then separated in a centrifuge.

There are other methods for extraction of pectins; but these are considered to be laboratory procedures rather than industrial. Two of these are McCready et al. (1952) versene method and Kertesz et al. (1950) pectic fractions method.

Properties of Pectins

Pectins are carbohydrate derivatives, composed mainly of anhydrogalacturonic acid residues, with arabinose, galactose, sorbose, rhamnose and other molecules either attached to the chains of anhydrogalacturonic acid units or in conjunction with them. The carboxyl groups in pectic galacturonides are either free or partially esterified with methyl groups or form salts with various cations.

According to the definitions, all pectic substances contain a large proportion of polygalacturonic acids which are built

from anhydrogalacturonic acid units. The simplest pectic structure is pectic acid, or tetragalacturonic acid (Myers et al., 1934), which has enough components to attain colloidal properties. Most polygalacturonic acids (pectic acids) are greater in units than the minimal tetragalacturonic acid structure. From a study of the physical properties of pectic substances, Meyer et al. (1930) assumed that pectic acids are built from galacturonic acid residues coupled into long chains with glycosidic linkages. Corbeau (1933) and Burgers (1933) concluded that the molecule must be very much elongated. This was proven by Henglein et al. (1936) by preparing nitropectin and finding that it shows properties similar to nitrocellulose.

Adjacent galacturonic acids in naturally occurring polygalacturonic acids are coupled through a β-1,4 linkage (Hirst, 1942). One of the best recent reviews on protopectin chemistry is that of Joslyn (1962). Although quite a large amount of knowledge has been accumulated about the pectin molecule, there are some features about it that cannot be explained, and these appear when pectin is extracted under different conditions or from different sources.

Joslyn et al. (1963), when preparing alcohol-insoluble solids from apple tissues and using different solvents, found marked differences in extractability of pectins present in marcs prepared in various ways from the same lot of apples. Enzymatic browning during preparation drastically decreased the solubility of pectins, but did not alter the composition of the pectins extracted. They also found that the solubility

of pectins in fresh or dried commercial apple pomace was markedly lower than that from carefully prepared non-oxidized apple tissue; and that pretreatment of browned marc preparations with chlorous acid bleached the marcs, yielded colorless pectins, and increased yields. The yields also differed when a variety of solvents were used. Griffin et al. (1946) found that treating apple tissues with ascorbic acid or hydrogen peroxide brought about a decrease in the total pectic constituent but an increase in water-soluble pectic materials. These changes are similar to those that occur during maturation and softening of apples.

Sinclair et al. (1949) separated the whole peel of Valencia oranges and the albedo of Navel oranges into alcohol-soluble and alcohol-insoluble fractions by extraction of the fresh material with hot 80% ethanol. They found that the alcohol soluble was 55 to 57% of total dry weight, while the alcohol insoluble was 42 to 44%. Ting et al. (1961) analyzed the carbohydrates of orange and grapefruit peel. They found that the alcohol soluble solids were composed of about 80% total sugars with glucose, fructose, and sucrose being the main sugars present. The alcohol insoluble solids were separated into pectic substances, hemicellulose, and cellulose fractions by extracting with different solvents. The pectic substances upon hydrolysis yielded arabinose, galactose, and galacturonic acid. On the average only between 53 and 70% of the alcohol-insoluble solids were recovered as carbohydrates in the peel of various citrus varieties. Rouse (1953) studied the distribution of total pectin in component parts of citrus fruit. He found that the

pectic content on a dry solid basis was found to be highest in the rag (segment membrane) of Dancy tangerine, Pineapple, Temple, and Valencia oranges, and Duncan grapefruit. In the Hamlin orange pectin was highest in the albedo and in the Marsh Seedless grapefruit pectin was highest in the flavedo. The pulp content of citrus juice consists mainly of rag and juice sacs, both of which contain a high percentage of pectin.

Evaluating the pectin in component parts of Valencia oranges (Rouse et al., 1962), Pineapple oranges (Rouse et al., 1964), and Silver Cluster grapefruit (Rouse et al., 1964 a), Rouse and co-workers found that the order of component parts for jelly units from highest to lowest was membrane, peel, and juice sacs. In the case of grapefruit they judged the residue a better source of raw material for the manufacture of pectin than that from oranges.

Three important factors that characterize pectins are:
1. The molecular weight or degree of polymerization of the pectin molecule.- The colloidal nature of pectic acids is believed by some to be due to the large linear molecules, while others feel that these polygalacturonic acid units are further combined among each other or perhaps with cellulose exclusively through calcium linkages.

An X-ray study conducted by Palmer et al. (1945) yielded some information on the special structure of pectic acid fibers. Later Palmer et al. (1950) concluded that the polygalacturonic acid chains have a two-fold screw symmetry and that the interchain separation per carbon atom in the ester chain is 0.78 Å. At the

same time Alfrey et al. (1942) saw no reason to assume that the shape of the pectic acid macromolecules is constant, and that the solvent or other external conditions might determine the prevalent shape of the polymer molecule.

The molecular weights of pectic substances from various sources have been the subject of numerous investigations. The results are influenced by the manner of extraction and method of measurement of molecular weight. The molecular weights which have been recorded range from about 10,000 to 400,000; and an examination of the literature shows the disharmony between the results of different authors. Some authors, such as Heri (1962), reported the presence of three fractions with a definite molecular weight in a sample of pure citrus pectin. One thing that most authors agree upon is the importance of molecular weight regarding the performance of pectic substances.

Pectins and other polysaccharides may be coagulated by the addition of organic and inorganic substances, especially electrolytes. The flocculation of pectins is governed by many factors. The susceptibility of pectinic acids to coagulation increases with the size of the molecule and decreases with degree of esterification, as reported by Haas-Schulz (1951) for apple pectins. He reported that pectin with a degree of esterification of 60% can be coagulated by calcium when the average molecular weight is above 14,800.

Protopectin and covalently linked pectinic acids show a limited swelling ability in water. The degree of swelling is dependent on the structure of the network, the molecular weight, the degree of esterification, the presence of side chains, pH

and the presence of salts in the surrounding medium. The swelling of pectic substances is enhanced by increases in molecular weight and degree of esterification. According to Henglein et al. (1949) the swelling power of dead vegetable tissues is decreased with diminishing degree of esterification and polymerization of the pectic substances in the tissues.

Pectins should be completely dispersed to obtain their maximum jellying power or to yield maximum viscosity. Dispersibility can be promoted by the presence of very small amounts of suitable metal ions such as aluminum, copper, iron, nickel, and chromium or by varying the manner of manufacturing, especially drying (spray dried pectins proved to dissolve more easily than the other pectins). Dispersibility is believed to be a function of polymerization.

Viscosity determination of pectic solutions has often been used to calculate their molecular weight, according to the principles given by Staudinger (1932). The viscosity of a solution can be characterized by the related function of specific viscosity. Many experiments on factors governing the viscosity of solutions of pectates, pectinates and pectinic acids have been made (Deuel et al., 1957; Lotzkar et al., 1946; Schultz et al., 1945). The higher the molecular weight, the greater the viscosity. Usually, reduction in the degree of esterification is accompanied by a drop in viscosity, however, when comparing the viscosity caused by high-methoxyl pectinic acids with their low-methoxyl derivatives it is necessary to take into account that during saponification, some breakdown

of polygalacturonic chains may occur. Generally, the viscosity of water solutions of pectinic acids is dependent on molecular weight, degree of esterification, presence of electrolytes, pH and concentration.

The mechanism of jellification has been discussed by several authors. Gels are commonly regarded as a two phase system with a high degree of interface between a continuous, or at least intermeshed, system of solid material holding an aqueous (or other solvent) phase which is also continuous or finely despersed. It has also been shown that there is a strong relation between jellying power and degree of polymerization. Olsen et al. (1939) reported that molecular weights of about 40,000, 100,000 and 175,000, respectively, corresponded with poor, moderate and very good jellying powers. Side chains and components covalently linked to the polygalacturonic chains disturb the relationship between molecular weights and jellying power. Doesburg et al. (1959) has studied the setting temperature and times of pectin gels. Hinton (1950) has shown that the time and temperature at which setting occurs is influenced by the cooling rate but that it is a determinate property of the pectin, which might be related to degree of esterification or polymerization.

2. Degree of esterification.- Properties such as mobility in an electrophoretic field, ionic bonding, or gelation in case of low methoxyl pectin are governed by the degree of esterification. The measure of esterification of pure polygalacturonic acids may be indicated by the methoxyl content or by the degree of esterification which represents the number of esterified carboxyl

groups calculated as the percentage of the total number of galacturonic acid units. When the carboxyl groups in pure polygalacturonic acids are all esterified, the methoxyl content is 16.32% and the degree of esterification is 100%. The upper limit of methoxyl content of pectinic acids, extracted from natural sources, is seldom higher than about 13.5%. Vollmert (1950) has shown that methylated pectinic acids can be prepared by treatment with diazomethane. A completely non-esterified pectic acid has been recently isolated by Anderson et al. (1961).

The kinetics of the deesterification of pectin seem uncertain (Speiser et al., 1945), although Merril et al. (1946) have shown it to be a first order reaction. They also found that the rate of degradation to the rate of ester hydrolysis of pectins in aqueous solutions is decreased by lowering the temperature or by the presence of acids. Most added neutral salts decrease the pH and increase the rate of deesterification of pectin solutions at their natural pH. Changes in cations usually affect the pH and rate of deesterification more than changes in anions except in the case of sulphates and bivalent cations which are more effective than monovalent. The influence of added salts is due mainly to their effect on pH. However, specific ion effects are found and these are due mainly to interaction of the carboxyl groups of the pectin with cations.

Hills et al. (1946) disagree with the above and stated that

the deesterification of pectin by tomato pectinesterase follows a zero order reaction for the initial 40 per cent rather than a first order reaction.

Deuel et al. (1957) also studied the kinetics of saponification of pectins and found that the kinetics of the alkaline saponification of the methyl ester of polygalacturonic acid cannot be described by a rate constant for second order reactions. However, the saponification of the methyl ester of galacturonic acid obeys this law. They feel that the unusual behavior of pectin seems to be caused by the increasing negative charge of the chain molecules during saponification. Therefore, the approach of negative hydroxyl ions becomes more difficult. They conclude that many properties of substances are highly dependent on their degree of esterification of the carboxyl groups which causes an increase in water solubility, dissociation of the acidic groups, viscosity, birefringence of flow, swelling, resistance toward electrolyte coagulation and alkali lability. All these changes in properties may be explained by a decrease in charge density, a stretching of the chain molecules, or the screening effect of bulky side groups.

Schultz et al. (1945) noticed a difference in pectinic acid deesterified with enzyme and pectinic acids deesterified with acid or alkali. Pectinic acids deesterified with citrus pectinesterase formed weaker calcium gels than pectinic acids of the same methoxyl content prepared with alkali. Their hypothesis in explaining this phenomena is based upon the fact that

the esterase deesterifies portions of the galacturonide chain, while acid and alkali act in a random manner.

Smit et al. (1967) separated pectin fractions on diethylaminoethylcellulose columns using increasing concentrations of sodium dihydrogen phosphate solution as the eluent. The fraction eluted with the lowest concentration of sodium dihydrogen phosphate had the highest methoxyl content, and later fractions, obtained with progressively increasing sodium dihydrogen phosphate concentrations, showed progressively lower methoxyl values. The percentage esterification and equivalent weight also decreased in a similar manner. This decrease in methoxyl content from one fraction to the next was accompanied by an increase in setting time and jelly grade.

3. Association of pectic compounds with other possible components.- According to Joslyn (1962) it has been impossible to prepare protopectin in an unchanged form from plants. For this reason the present knowledge of the pectic substances has been derived from observations on pectic substances obtained by extraction with various reagents under different conditions. Doesburg (1965) reports that the crude alcohol precipitate of pectic substances extracted from plants may contain as much as 50% non-uronide matter, determined as ash, nitrogenous constituents, polysaccharides (mostly glucosans and hemicelluloses) and other substances. According to the literature the amount of covalently linked non-uronides seems to vary considerably. Bishop (1955) reported that pectic substances from sunflower heads, extracted under very mild conditions, appeared to be pure galacturonides. McCready et al.

(1960), when analyzing purified pectinic acids from several fruits, carrots, sugar beet and pea pods, after partial acid hydrolysis, found various sugar contents ranging from 8 to 25%. It is believed that the amount of non-uronide matter in pectic substances is influenced by the manner of extraction and precipitation. The results of several experiments indicate that non-uronide constituents are covalently linked to the polygalacturonic chains. Jansen et al. (1949), Palmer et al. (1945), and Lineweaver et al. (1961) concluded, respectively, from experiments on methanolysis, X-ray analysis, and enzyme activity that some of their components are present in the chains, whereas, Hills et al. (1946) and other authors have pointed out that these constituents are attached as side chains.

According to Speiser et al. (1945) arabinose and galactans are bound to the pectic substances from flax and apples, respectively. As reported previously, McCready et al. (1960) demonstrated arabinose, galactose, rhamnose and xylose in purified pectic substances from various sources.

From the work of Vollmert (1947) and Henglein (1950) it can be concluded that a rather high percentage (6%) of acetyl groups can be found in sugar beet pectins, whereas, the acetyl content in citrus pectins is low.

Henglein et al. (1949) claim the occurrence of phosphoric acid in ester or ionic bondage, and that it may play a part in protopectin structure.

Newbold et al. (1952) believe that the non-uronide material apparently reduces the specific optical rotation of pectic acid

preparations, as the specific rotation is proportional to the percentage of uronide matter.

The rehydration and swelling of pectins will vary according to the ingredients associated with it. Baker et al. (1952) showed that high methoxyl pectins, in a pectin-cellulose inert ingredient mixture containing 20% cellulose, decreased the rehydration swelling of the cellulose at 26° C, but caused greater swelling at 90° C. They also formulated a "synthetic" fruit mixture containing cellulose, sugars, salts, acid, and high methoxyl pectin in proportions similar to the average analysis of miscellaneous fruits and showed very little rehydration swelling at either 26° C or 90° C but rehydration did occur when the pectin had been partially deesterified with pectin methylesterase prior to dehydration.

From the discussion on structure it is clear that pectic substances show a very great heterogeneity, which is a result of variations in molecular weight, amount and distribution of methoxyl and acetyl groups, and quantity and distribution of other non-uronide materials attached to the polygalacturonic chains as well as irregularities in these chains.

Gelation

Jellying power is an important property of pectins, and is the main reason for their commercial production. When a sufficient amount of high methoxyl pectin is present for the formation of a gel, three other conditions must be fulfilled:

(a) the electro-static repulsion between pectin molecules has to be decreased by depression of dissociation of carboxyl groups, (b) sucrose or similar compounds have to be added in sufficient amount, (c) hydrogen ion of a correct range (pH) must be present.

The jellying power of pectins is related to their sugar carrying power; jelly grade is the proportion of sugar which one part of pectin will gel under standard conditions.

Solms (1956) has shown that gelation occurs mainly through hydrogen bond formation. Several neighboring hydroxyl groups on different macromolecules form zones of attachment. Because the hydroxyl groups are linked to a more or less rigid backbone chain, the fine structure of this chain is of great importance for arranging these groups in complementary position, forming functional surfaces. He also noted that groups in equatorial positions seem to be more readily accessible for chain-to-chain reactions than groups in axial positions. Carboxyl groups do not seem to share directly in the formations of the junction zones. They are important as regulators of the potential for the chain molecules. This hypothesis may help to show that the mechanism of gelation is a very specific reaction.

In the case of calcium polyuronate gels, Sterling (1957) showed a more or less selective uniplanar and a uniaxial orientation according to the evidence of X-ray diffraction patterns in the three major axes. He interpreted his result to mean that polyuronate gels have relatively strong polyuronate to polyuronate bonds in the radial direction; and these supplement the weaker polyuronate-water bonds. Bennison et al. (1939)

explained the relationship between jellification and composition of various pectins. They noted that there is little relation between chemical composition and jelly strength, but a high urone content denotes satisfactory jellification. They also noted that methoxyl content is not a criterion of jelly strength, and that esters of pectic acid prepared by methylation failed to give jellies. In short, he states that composition, primarily molecular size, and method of preparation are the vital factors with respect to gelation.

Speiser et al. (1946) agree with the above in saying that the strength of hydrogen-bonded gels are primarily determined by molecular weight, but substantially independent of degree of esterification and method of deesterification. They add that the strength of ionic-bonded gels is less affected by molecular weight than hydrogen bonded gels; but is strongly dependent on degree of esterification.

Joseph et al. (1949) noticed that pectinates deesterified with acids, fixed alkali or dilute aqueous ammonia systems and enzymes, have essentially the same structure but exhibit characteristic differences in such properties as viscosity, calcium sensitivity, and gel formation. Hills et al. (1949) explain the difference in gelation characteristics of enzyme and acid deesterified pectic acid on the basis of fundamental differences in composition and structure of the two products. Acid catalysis causes the similtaneous removal of methyl ester groups and non-galacturonide materials. Enzyme deesterification neither

completely removes all of the methyl ester groups nor does it affect the non-galacturonide materials. For this reason the enzyme-prepared pectinic acids are more readily soluble in aqueous solutions.

Hinton (1940) believes that another factor which controls jelly formation is the ionization of the pectin molecule, and on the assumption that jelly formation is only participated in by molecules in the non-ionized condition; that it is shown that the concentration of such non-ionized pectin must exceed a certain solubility, or saturation limit, which varies with the total solids concentration of the mixture. This proportion varies with the nature of the buffer salt present and with the degree of esterification of the pectin. Harvey (1950) added a slight modification to Hinton's hypothesis to provide a quantitative explanation of some of the differences in jellying behavior caused by different metallic buffer salts. The modified theory leads to a simple conception of effective ionization and this in turn is used to account for some of the differences in jellying behavior brought about by alterations in the degree of esterification of the acid groups of the pectin molecule.

Radiation

The use of radiation as a means to pasteurize food products has become of widespread interest in the past fifteen years. Irradiation of fruits and vegetables has been a controversial subject, probably from the failure of some investigators to

recognize the living nature of fruits and vegetables (Maxie et al., 1965). The effect of radiation on pectic substances is important because radiation-induced changes in texture must not make the commodity susceptible to impact and vibration injuries during shipment by rail or truck.

Eukel et al. (1959) described several aspects of a program to bring shelf-life extension by low-dose irradiation with a high energy electron beam from a linear accelerator to develop a commercial process for certain fresh foods available in abundance on the West Coast.

The effect of ionizing radiation on naturally occurring polysaccharides has been studied by several scientists. Lawton et al. (1951) and Saeman et al. (1952) have studied cellulose, Price et al. (1954) and Ricketts et al. (1954) have studied starch.

The changes that were observed to affect adversely processed products were studied by numerous investigators (Desrosier, 1960; Francis et al., 1960; Franceschini et al., 1959; Huber et al., 1953; Lukton et al., 1956; Markakis et al., 1959).

In studying the effect of ionizing radiation on plant tissues, Boyle et al. (1957) calculated the dosage required to cause a 50% decrease in firmness to be from 123 x 10^3 to 834 x 10^3 reps for apples and from 671 x 10^3 to 2,310 x 10^3 reps for carrots. Massey et al. (1964) reported that in the case of apples, there is a softening effect by radiation dosage in excess of 10 krad, but in storage, irradiated fruit softened at a much slower rate than

did non-irradiated fruit. They also state that softening was positively correlated with change in pectin. Al-Jasim et al. (1965) indicated that calcium dislodgment from plant tissues by irradiation dosages of 200 to 600 krad may contribute to the radiation-induced softening of fruits and vegetables.

In a review of the status of irradiation effects on citrus fruit, Dennison et al. (1966) reported a sizable increase in total pectic substances in the juice of Valencia oranges and Duncan grapefruit. Higher juice yields with increased viscosities were obtained from irradiated Pineapple oranges and Duncan grapefruit. Rouse et al. (1966) also found an increase of 66 to 488% in water-soluble pectin when citrus fruits were exposed to 300 krad. Dennison et al. (1966) and Grierson et al.(1965) noticed severe peel injury on both oranges and grapefruit resulting from irradiation treatments, but that Temple oranges were the least susceptible. Dwight et al. (1938) concluded that irradiation of apple pectin by soft X-ray profoundly reduced the viscosity of the resulting sol. Wahba et al. (1962) showed that low-dose gamma irradiation of dilute aqueous pectin solutions, after appropriate adjustment of pH, leads to the formation of thermoreversible gels. Phillips (1961) explained the above phenomena by stating that high-energy radiation unexpectedly produces cross-linking in some cellulose compounds; and that highly viscous solutions give limited freedom of motion to polymer chains and that high-energy radiation degrades these systems until the fall in viscosity permits free, bimolecular

coupling reaction, at which point, the system immediately gels.

Results of an electrophoretic study on irradiated pectins, conducted by Skinner et al. (1960), showed that no extra components are produced upon irradiation and that there was no change in the mean mobility of the pectin boundary or in the mobility distribution about the mode. These results indicate that the effect of radiation was to produce random hydrolytic fissure of the glycosidic linkages along the length of the pectin molecule.

Somogyi et al. (1964) extracted pectins from irradiated pears and peaches and their results revealed only minor differences in respect to their anhydrouronide, acetyl content, and degree of esterification.

Kertesz et al. (1956) studied the effect of radiation on pectin powder, pectin solutions and pectin gels. They found that through viscosity measurements of subsequently made solutions, pectin powder is degraded by electron bombardment or gamma radiation of 50,000 reps. In solution, they found that pectin is degraded by gamma radiation of 8,300 reps. They also stated that sucrose, glucose and fructose added to the solution protected the pectin, and that protection can be complete when the sugar concentration was high enough and the pH of the solution low enough to allow gel formation. Kassem et al. (1967) found a definite change in irradiated gel characteristics as measured by a modified attachment to the Kramer Shear Press.

Studying the effect of gamma radiation on the yield, jelly grade, jelly units and methoxyl content of pectin from citrus fruits, Rouse et al. (1968) found that irradiation increased the

yield but both jelly grade and jelly units decreased. Methoxyl content also decreased in pectins extracted from peel.

Determination and Characterization of Pectic Substances

For a long time it has been suggested that a pectin determination is somewhat empirical. Today there are relatively accurate methods for the determination of pectin amounts and properties.

One of the earliest classical methods for the determination of pectic substances was that of Carre-Haynes calcium pectate method. Nevertheless, in later years much evidence has shown that, in many cases, appreciable amounts of admixed ballast materials are entrapped in the meshwork of pectic material which causes erroneously high values when determined as calcium pectate (Ahmed et al., 1958). The determination of uronide content by decarboxylation of uronic acids by boiling with 12% hydrochloric acids, according to the modifications of McCready et al. (1946) and the micromethod of Tracey (1948), is often used. Colorimetric methods are also used for the determination of the uronide content. As compared with the method based on anthrone (Helbert, 1956 and 1957), naphthoresorcinol or dinitrobenzoic acid, the carbazole method of Dische (1950) has found most wide application. McComb et al. (1952) have found that the color reaction with carbazole was more intense when the pectins were first deesterified with alkali. Stark (1950) modified this method for the determination of pectic materials in cotton. The chemistry of the procedure

was developed by identification of 5-formylpyroracemic acid, a compound formed on dehydration and decarboxylation with sulphuric acid which actually reacts with carbazole.

There are many other methods for the determination of pectic substances, such as the method of Okamasu (1956), Almendinger et al. (1953), Fellers et al. (1932), McCready et al. (1951).

Dietz et al. (1953) reported on a rapid method for estimating pectic substances in citrus juices suited to routine control work. Later this method was revised by Rouse et al. (1955).

The equivalent weight and degree of esterification is determined by titrimetric methods as described by Owens et al. (1952).

Since the most important factor about pectin is its ability to form gels, jellying ability is known as 'pectin grade', and it is the number of pounds of sugar that one pound of pectin will gel in a standard 65% sugar jelly. Since pectin gels are plastic or elastic solids, their firmness can be indicated by the extent to which they sag when turned out of a container or by their resistance to penetration by a variety of instruments. Therefore, Christensen (1954) divided the methods for the determination of gel strength into large groups.

Group I. The elastic limit of the jellies is exceeded and the jellies rupture; measurement of breaking strength.

a. Succharipa's jelly disc method.

b. Fellers-Claque's penetrometer method.

c. Luers-Lochmullers' 'Pektinometer' method.

d. Delaware Jelly Strength Tester (Tarr-Baker).

Group II. Deformation of the jellies within the limit of elasticity.

a. Bloom gelometer.

b. Cox and Higby's sag method.- This method and the Delaware tester have attained wide popularity for grading pectins in this country.

c. B.A.R. Jelly Tester (Campbell).

d. Saverborn cylindrical torsion method.

MATERIALS AND METHODS

The material used as a pectin source was the peel, rag and cores of Valencia oranges discarded from an FMC In-Line Juice Extractor at the University of Florida Citrus Experiment Station, Lake Alfred, Florida. One hundred pounds of material were collected.

The material was comminuted in a Fitz Mill Comminuting Machine Model D at slow speed using a screen with one inch square holes. This permitted the material to be sized without pulping. Fifty pounds of material, at a time, were added to twenty-five gallons of water and heated to 90° C in a steam jacketed kettle for five minutes, after which twenty-five gallons of water at room temperature were added to lower the temperature to 60° C. Hot water was used to remove water-solubles from the material and to inactivate the pectinesterase enzyme. Two more washings with water at room temperature were made to remove the water-soluble fractions. Between washes, excess water was removed by running the material over a shaker screen. After the material was washed, it was reduced to 86% moisture by pressing in a hydraulic press under 164 psig. This will be referred to as the wet residue.

One-half of the pressed material was dried in a cabinet drier at 60° C with an air velocity of 60 ft^3/min. It was left in the drier for twenty-four hours until it reached 6% moisture. This portion of the sample will be referred to as the dry residue.

The dried and wet samples were divided each into eleven portions and each portion was packed into a plastic bag and irradiated.

Both electrons and gamma rays have been used for the processing of food products. Gamma rays have a greater penetrating power than fast moving electrons. The thickness in water required to reduce the intensity of the beam to one-half of its initial intensity is about four inches for the 1.1 and 1.3 Mev gamma rays of Co^{60} and slightly more than 3 inches for the 0.67 Mev gamma rays of Cs^{137}. On the other hand electrons are preferable for their higher dose rate. The samples were irradiated in the Mark III Cs^{137} Food Irradiator located at the Food Science Department, University of Florida, Gainesville. Dose rates for this unit were 2 krad/min for the center cell. The samples were placed into the irradiator chamber and lowered down to the source plaques for a period of time calculated to give the required dosage for the particular treatment. Controlled temperature in the chamber was 55° F, with a continuous flow of air at 20 liters/min during the irradiation period. The doses were from 0 (control) to 1,000 krad, with 100 krad increments. The dried irradiated samples were stored in the laboratory at room temperature until analyzed while the wet samples were stored frozen at 0° F to

prevent degradation of pectin.

Each sample was analyzed for percentage yield of pectins and jelly grade. When these two results are multiplied, they equal jelly units of the residue. It indicates the amount of sugar that can be jelled by one unit residue. Methoxyl content and purity of the pectins, as anhydrogalacturonic acid (AGA), were determined and methoxyl content on AGA basis was computed. Relative viscosity was also measured for each of the forty pectins extracted from the residues.

Extraction

Pectin was extracted from each sample using water only and water plus Zeo-Karb H. All extractions were made for one hour at 90° C with a ratio of residue to water, 1:80. In the samples extracted with Zeo-Karb H the ratio of residue to Zeo-Karb H was 8:10. These ratios were applied on dry-weight basis of the prepared residues. The Zeo-Karb H extraction is equivalent to an acid extraction of pH 2.4 to 2.8. The ratio of water to solids and vigorous agitation were maintained throughout the extraction period. The mixture was then cooled to 60° C by circulating tap water around the flask. After centrifugation at 2400 rpm for 10 minutes the liquid was decanted; solids from the centrifugation were again extracted with a small amount of distilled water at 40° C, recentrifuged and decanted. The liquids were combined and filtered through a prepared Buchner funnel with gentle suction. The prepared mat on the Buchner funnel consisted of Cenco No. 13255 filter paper coated with a mixture of 10 g each of Standard Super Cell and Hyflo. The filtrates from the

water extraction were acidified to a pH similar to the filtrate from the Zeo-Karb H extraction (approximately pH 2.75). The filtrates were precipitated in 1 volume of acetone, washed 2 times with 70% ethanol, and once in 95% ethanol. The washed precipitates were dried under vacuum at 60° C for 12 hours. These forty-two dried alcohol precipitates were used in the following determinations:

Yield

Yield was calculated from the alcohol precipitate on dry residue basis.

Jelly Grade

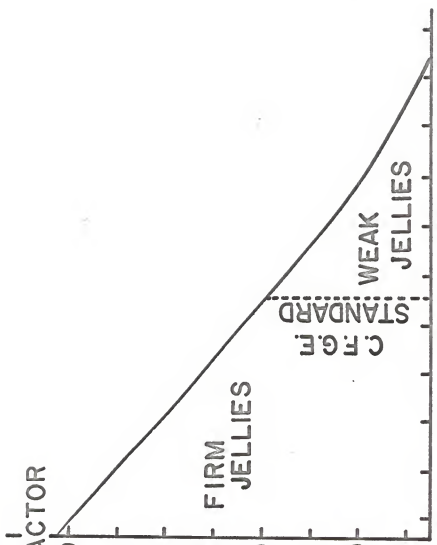
The method used for determining jelly grade was that of the IFT Committee or Pectin Standarization, with slight modifications (1959).

A 2% weight pectin solution was prepared from each of the forty pectins extracted. The weight of the pectin used was calculated by dividing 650.0 g (sugar) by the value of an assumed grade for the pectin. Relative viscosity of a 0.5% pectin solution was the criteria used to assume a pectin grade. The amount of sugar 650.0 g minus the weight of pectin was used. The correct amount of pectin in solution was transferred to a stainless steel pan containing a stainless steel stirrer. Then a portion of 410 ml of water were added. . The sauce pan was then placed on a hot plate resting on a torsion balance and heated until the contents came to a full rolling boil, stirring was continuous during this period.

Sugar was added and stirring and heating continued until the sugar was dissolved. Heating was continued until the net weight of the jelly batch was 1015 g. The entire heating time of the jelly was from 5 to 8 minutes. The 1015 g batch was removed from the balance, allowed to sit undisturbed, the pan tipped, and any foam was skimmed off. When the temperature of the batch reached 95° C, it was poured quickly into three previously prepared Ridgelimeter glasses, each containing 2 ml of tartaric acid solution (48.8 g of tartaric acid crystals made to 100 ml). Scotch brand masking tape, 3/4 inch wide, was used to make "sideboards" on the glasses. About fifteen minutes after the glasses were filled they were covered with metal lids and stored 20 to 24 hours at 25° C. After the storage period, the lids were removed and the tape strips torn off. With the use of a cheese cutter, the layer of jelly projecting above the top of the glass was removed and discarded. The jelly was turned out of the glass in an inverted position on a plate glass that accompanies the Ridgelimeter. A stopwatch was started as soon as the jelly was on the glass plate. Two minutes after the stop watch was started, the point of the micrometer screw was brought just into contact with the top jelly surface. Percentage sag was read directly off the Ridgelimeter. Ridgelimeter readings on different glasses from the same batch should not vary more than 0.6.

The relationship between "assumed grade" and the factor to obtain "true grade" is represented by a very slightly S-shaped curve shown in Fig. 1.

Figure 1. Exchange Ridgelimeter calibration curve for grading pectins (California Fruit Growers Exchange).


FACTOR
1.20
1.15
1.10
1.05
1.00
0.95
0.90
0.85
FIRM JELLIES
C.F.G.E. STANDARD
WEAK JELLIES
19
21
23
25
27
29
PERCENT SAG OF JELLY

Purity or Percentage Anhydrogalacturonic Acid (AGA)

Purity of pectin was analyzed according to the method of Rouse et al. (1955). Ten g of 1% pectin solution were weighed and made to 500 ml. Twenty-five ml of the solution were pipetted into a 100 ml volumetric flask, 5 ml of 1.0 N sodium hydroxide were added to saponify the pectin to sodium pectate, the volume was brought to 100 ml, and left to stand 30 minutes. One ml aliquot of the saponified solution was pipetted into a large test tube (25 x 200 mm) and 0.5 ml of 0.1% alcoholic carbazole was added to it. One-half ml of 95% ethanol was added to the blank tube. A white flocculent precipitate formed in the sample tubes. Six ml of concentrated sulphuric acid were added to the tube with constant agitation forming a pink color. The burette was such that it delivered 6 ml of acid in seven seconds, to control the heat of solution. The tubes were then immediately placed in a water bath at 85° C for exactly 5 minutes, then removed, allowed to cool for 15 minutes, and the percentage transmittance was read on a Bausch and Lomb Spectronic 20, at 525 millimicrons.

Preparation of Standard Curve with AGA

A sample weighing 120.5 mg of galacturonic acid monohydrate (dried at 30° C) was transferred to a liter volumetric flask. One-half ml of 1.0 N sodium hydroxide was added and the volume brought to one liter. The solution was left standing overnight. This standard solution contained 100 µg of AGA per ml. Working standards were prepared from the above solution covering the range

of 10 to 70 μg of AGA per ml. The color was developed as described for the samples (Fig. 2).

Equivalent Weight and Methoxyl Content

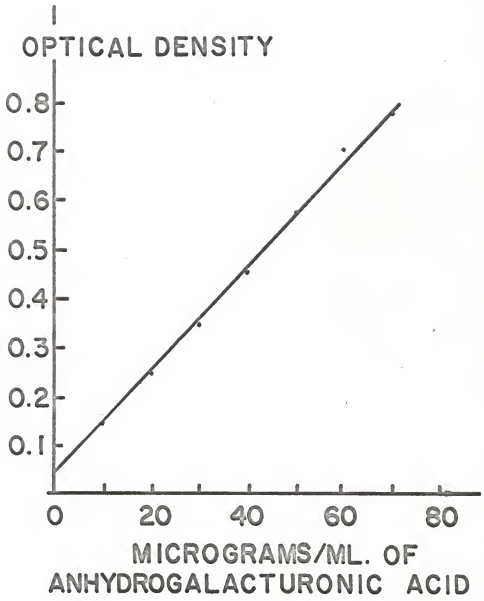
The methods used to determine equivalent weight and methoxyl content followed closely those used by Western Regional Laboratory (1951). One-half g of pectin was weighed, transferred to a 250 ml erlenmeyer flask, and moistened with 5 ml of ethanol. One g of sodium chloride and 100 ml of carbon dioxide free distilled water were added. Two drops of Hinton's indicator (1940) were used to indicate the end point. The pectin solution was slowly titrated against 0.1 N sodium hydroxide. Equivalent weight was calculated using the following formula:

$$\text{eq. wt.} = \frac{1{,}000 \times \text{wt of sample}}{\text{N} \times \text{vol. alkali}}$$

To determine methoxyl content, 25 ml of 0.25 N sodium hydroxide were added to the neutral solution titrated for equivalent weight. The solution was shaken and allowed to stand thirty minutes in a stoppered flask. Twenty-five ml of 0.25 N hydrochloric acid were added (or an amount equivalent to the base added) and the solution was titrated against 0.1 N sodium hydroxide. Methoxyl content was calculated using the following formula:

$$\%\ \text{MeO} = \frac{\text{N} \times \text{vol. alkali} \times 3.1}{\text{wt of sample}}$$

Figure 2. Standard curve for galacturonic acid.


OPTICAL DENSITY
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
20
40
60
80
MICROGRAMS/ML. OF
ANHYDROGALACTURONIC ACID

Relative Viscosity

Viscosity was measured on a 0.5% pectin solution at 25° C with a Fisher-300 viscosimeter from forty pectins extracted.

Statistical Analysis

The experimental design was a split plot and the data were analyzed using an analysis of variance and a Tukey's range test.

RESULTS AND DISCUSSION

Extraction and Yield

The non-irradiated residues extracted with Zeo-Karb H yielded 29.0% and 34.7% pectin for the dry and wet respectively (Fig. 3). Upon receiving 1,000 krad the dry yielded 31.1% (a 7.1% increase) and the wet yielded 37.3% (a 7.5% increase). The wet yielded more pectin than the dry because the wet residue was easier to commute and more surface area was exposed during extractions. The increase in yield that accompanied irradiation was not large because the Zeo-Karb H hydrolyzed most of the protopectin to pectic and pectinic acids and irradiation will not increase the yield.

Residues extracted with water exhibited very low yields at low doses (Fig. 4). The wet residue water extracted yielded from 12.9% pectin at 100 krad to 26.5% at 1,000 krad, or an increase of 105.4%. The dry residue water extracted yielded from 11.2% at 100 krad to 18.5% at 1,000 krad or an increase of 64.9%. Here again in both cases the wet residue yielded more than the dry.

Data for the 0 krad water extracted residues are not presented because yields were so low that large quantities of residue had to be used to give reliable results. Most of the water-soluble

Figure 3. Effect of gamma radiation on the yield of pectins extracted from Valencia orange dry and wet residue extracted with Zeo-Karb H.

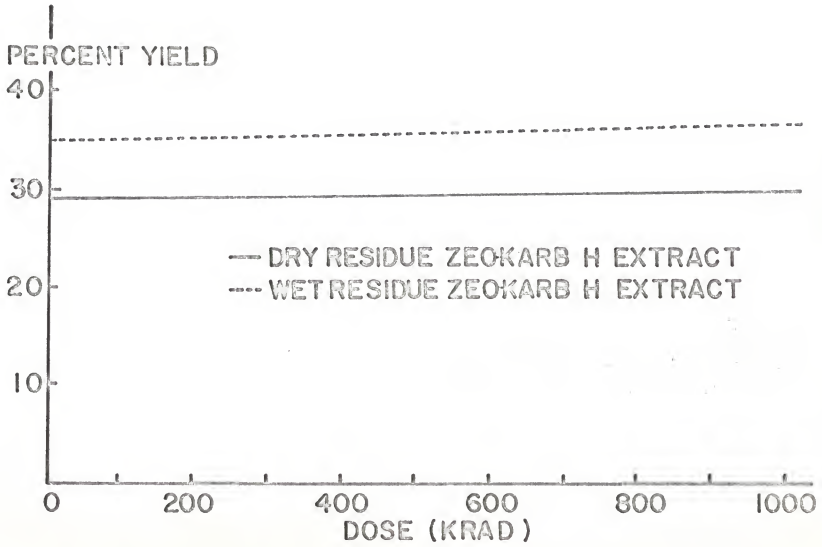
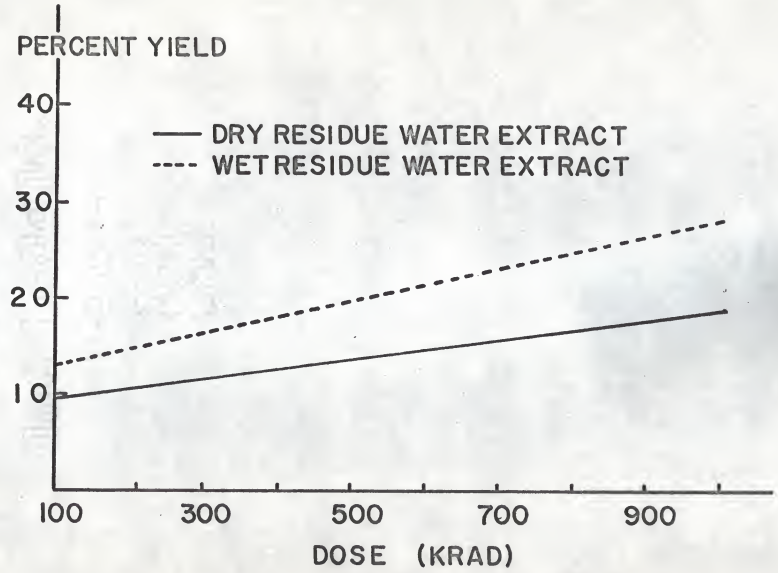

PERCENT YIELD
40
30
20
10
DRY RESIDUE ZEOKARB H EXTRACT
WET RESIDUE ZEOKARB H EXTRACT
0
200
400
600
800
1000
DOSE (KRAD)

Figure 4. Effect of gamma radiation on the yield of pectins extracted from Balencia orange dry and wet residue extracted with water.


PERCENT YIELD
40
30
20
10
DRY RESIDUE WATER EXTRACT
WET RESIDUE WATER EXTRACT
100
300
500
700
900
DOSE (KRAD)

pectinic acids naturally found in the residue were washed out during the washing of the material.

The difference in yield between the wet residue and dry residue extracted with water indicated that the effect of hydrolysis by irradiation was more severe on the wet material than the dry. This might have been due to the radiolysis of the water in the wet residue, and the production of primary active species which consist of hydrogen atoms and hydroxyl radicals. These free radicals could have induced a great variety of oxidation-reduction reactions. The difference between the wet and dry material might have been also due to an autolytic effect, which is higher in the dry than the wet residue. The wet residue gave 14.9% higher yield than the dry residue at 100 krad, and the difference becomes greater at higher doses. For example, at 1,000 krad the wet residue gave 42.7% higher than the dry (Fig. 4).

The difference between the water extracted residue (Fig. 5) and the Zeo-Karb H extracted residue (Fig. 6) was large at low doses, due to the difference between the hydrolyzing effect of the Zeo-Karb H and the hydrolyzing effect of irradiation at low doses. This difference becomes smaller as yields of water extracts increased due to higher doses. At 1,000 krad the Zeo-Karb H yields 44.8% more pectin in the case of the wet residues and 68.1% more pectin in the case of the dry residues. These differences were due to the formation of insoluble calcium and magnesium pectinates. The basic advantage of using Zeo-Karb H is to solubilize

Figure 5. Effect of gamma radiation on the yield of pectins extracted from Valencia orange wet residue extracted with Zeo-Karb H and with water.

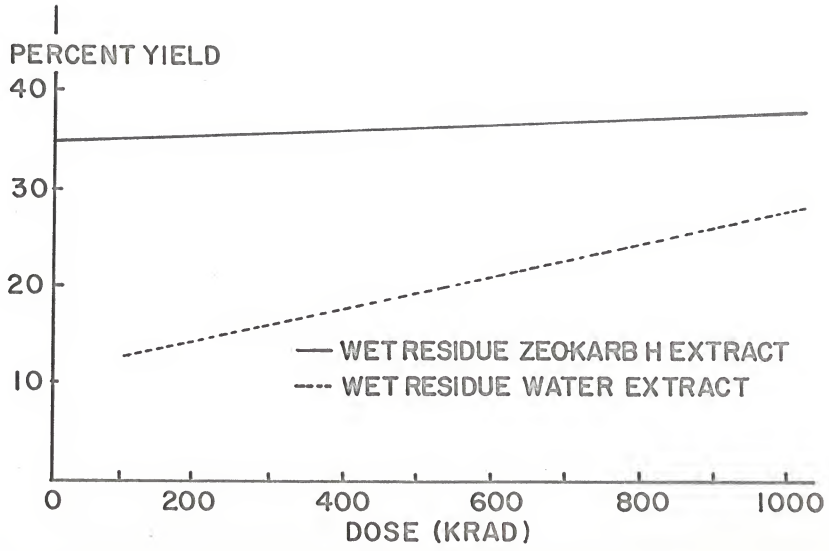
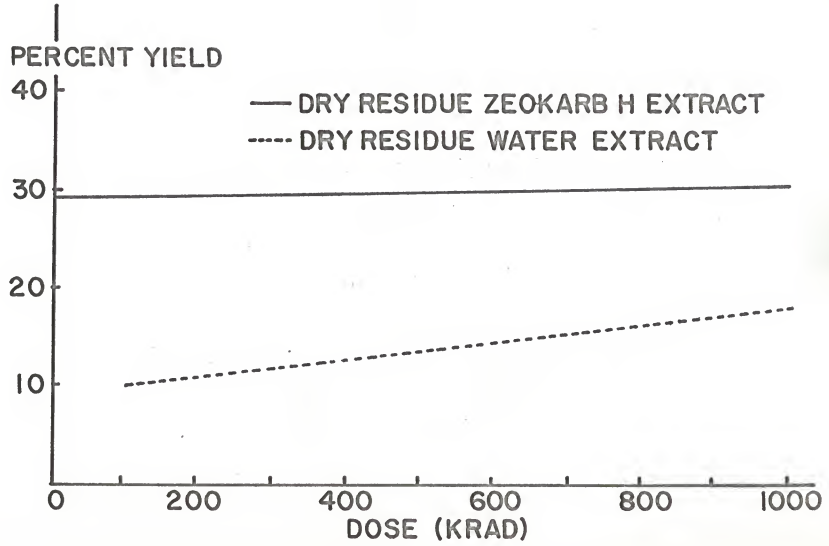

PERCENT YIELD
40
30
20
10
WET RESIDUE ZEOKARB H EXTRACT
WET RESIDUE WATER EXTRACT
0
200
400
600
800
1000
DOSE (KRAD)

Figure 6. Effect of gamma radiation on the yield of pectins extracted from Valencia orange dry residue extracted with Zeo-Karb H and with water.


PERCENT YIELD
40
30
20
10
0
200
400
600
800
1000
DOSE (KRAD)
DRY RESIDUE ZEOKARB H EXTRACT
DRY RESIDUE WATER EXTRACT

the calcium and magnesium pectinates by ion exchange to pectinic acids; this does not happen in the case of irradiation. Therefore, if irradiation was used to solubilize and extract pectins, the alcohol precipitates have to be washed with acid alcohol to lower the ash content, after which the acid has to be removed with further alcohol washings. Statistically it can be seen from Table 1 that both dose and extraction of the residues were significant at the 0.01 level, so was their interaction. From Tukey's test (Table 2) it was apparent that the significance was due to the water extracted samples where there was a significant change approximately every 300 krad.

In all extractions, yield represented pectinic acids and salts of pectinic acids; either native normal or acid pectinates or those that were solubilized from protopectin.

Jelly Grade

Pectins extracted from non-irradiated residues had a relatively low jelly grade because the residues were from Valencia oranges picked late in the season. Pectins extracted from fruit picked in March had a jelly grade of 337 (Rouse et al., 1968).

Zeo-Karb H extracted pectins (Fig. 7) and water-extracted pectins (Fig. 8) showed a drastic drop in jelly grade when the residues were irradiated from 100 to 1,000 krad. This was shown to a lesser degree by Rouse et al. (1968). It can also be seen that jelly grade decreased almost in a linear manner until it reached a minimum at 900 krad, after which it showed a

Table 1. Effect of gamma radiation on the yield of pectins extracted from Valencia orange residue.

Analysis of Variance

Source	d.f.	M.S.	F. ratio
Replication	2	2.44	1.19
Residue	3	2848.35	1382.70 **
Rep x Res	6	2.06	
Dose	9	62.73	74.48 **
Dose x Res	27	13.40	15.92 **
Error	72	0.84	
Total	119		

* Significant at 0.05 level
** Significant at 0.01 level

Table 2. Effect of gamma radiation on the yield of pectins extracted from Valencia orange residue

Tukey's Test

Dry Residue Water Extraction										
Dose	200	100	300	400	500	600	700	800	900	1000
Mean Yield (%)	10.63	11.21	12.66	14.40	15.09	15.12	15.70	16.19	17.63	18.50
Statistical Significance at 0.01 level										
Dry Residue Zeo-Karb H Extraction										
Dose	200	300	100	400	900	500	600	1000	800	700
Mean Yield (%)	28.99	29.13	29.52	29.85	30.76	30.80	30.83	31.05	31.07	31.88
Statistical Significance at 0.01 level										
Wet Residue Water Extraction										
Dose	100	200	300	400	500	600	700	800	1000	900
Mean Yield (%)	12.88	14.16	15.52	18.59	20.57	21.90	23.47	24.18	26.47	26.63
Statistical Significance at 0.01 level										
Wet Residue Zeo-Karb H Extraction										
Dose	600	400	300	500	200	100	700	1000	800	900
Mean Yield (%)	34.31	35.00	35.01	36.08	36.24	36.49	37.19	37.33	37.72	38.71
Statistical Significance at 0.01 level										

Figure 7. Effect of gamma radiation on the jelly grade of pectins extracted from Valencia orange dry and wet residue extracted with Zeo-Karb H.

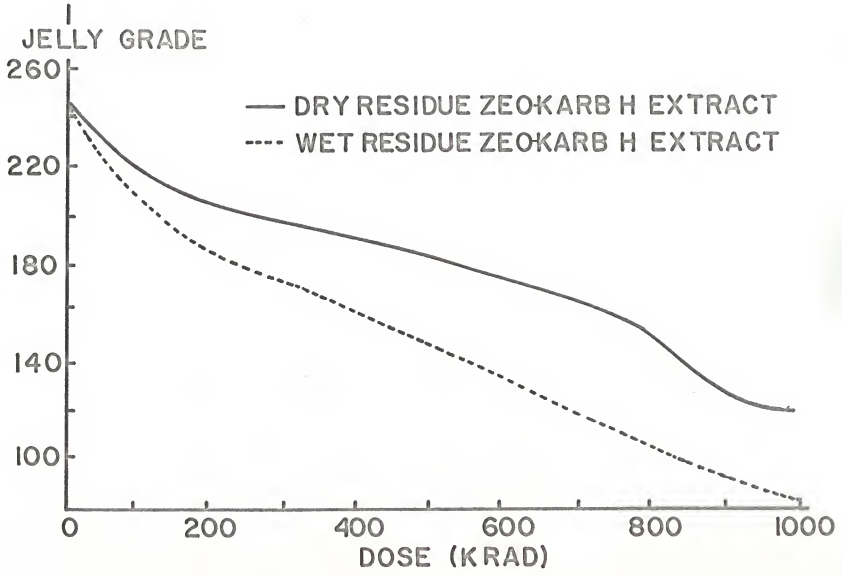
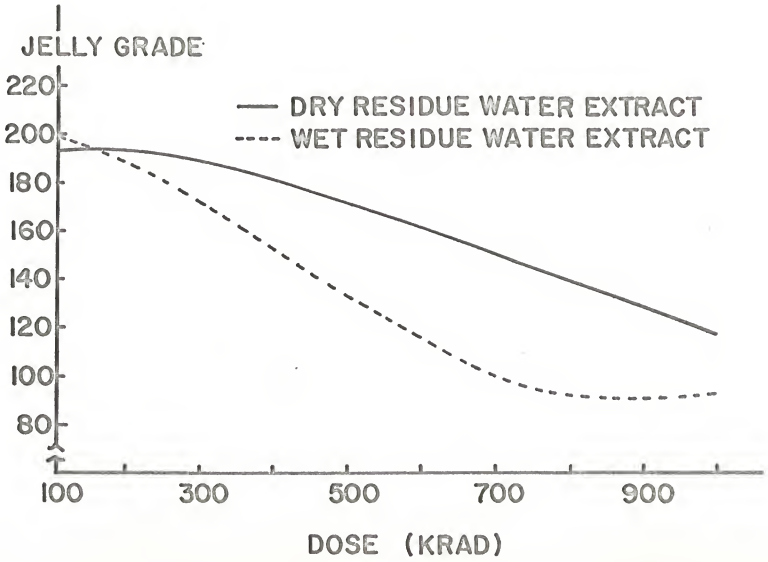

JELLY GRADE
260
220
180
140
100
DRY RESIDUE ZEOKARB H EXTRACT
WET RESIDUE ZEOKARB H EXTRACT
0
200
400
600
800
1000
DOSE (KRAD)

Figure 8. Effect of gamma radiation on the jelly grade of pectins extracted from Valencia orange dry and wet residue extracted with water.


JELLY GRADE
220
200
180
160
140
120
100
80
DRY RESIDUE WATER EXTRACT
WET RESIDUE WATER EXTRACT
100
300
500
700
900
DOSE (KRAD)

trend of leveling off. Jelly grade decreased 41.4% and 44.8%, respectively, for the dry residue water extracted and the dry residue Zeo-Karb H extracted (Fig. 9). In the case of the wet residues the drop in jelly grade was 51.4% for the water extracted and 58.1% for the Zeo-Karb H extracted (Fig. 10).

Jelly grade was lower in the case of the pectin extracted from the wet residue than it was from the dry residue using both water and Zeo-Karb H (Fig. 7 and 8). At 100 krad the jelly grade from the dry residue Zeo-Karb H extracted pectin was 4.4% higher than the wet residue. This difference became larger at higher doses and was 42.6% at 1,000 krad. The same effect was noticed in the pectin from water extracted residue, the jelly grade was 1.0% higher for the dry residue at 100 krad and 27.5% higher at 1,000 krad. This again showed that irradiation will affect the grade of pectin extracted from wet residues more than it will affect the grade of pectin extracted from dry residues. Pectins extracted from dry residue with Zeo-Karb H had a jelly grade which was 7.1% higher than the pectins extracted with water at 100 krad , while there was no large difference in the case of the pectins extracted from wet residues with Zeo-Karb H or water. This was because more protopectin was hydrolyzed in the case of the wet material than in the dry.

Table 3 shows that statistically there was a significant difference at the 0.01 level between doses and extraction of residues, and there was a significant interaction between doses and extraction of residues. From Tukey's test (Table 4) it can be seen that the difference was significant at almost every 100

Figure 9. Effect of gamma radiation on the jelly grade of pectins extracted from Valencia orange dry residue extracted with Zeo-Karb H and with water.

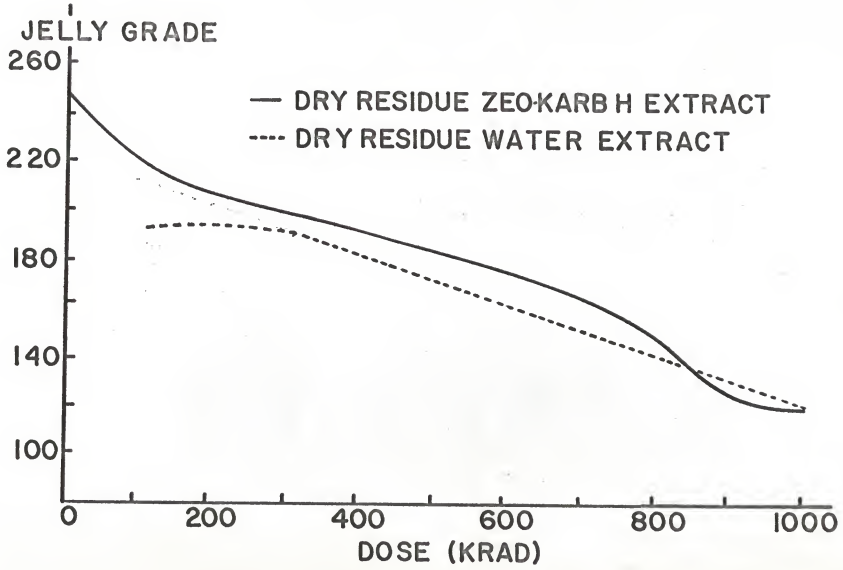

JELLY GRADE
260
220
180
140
100
0
200
400
600
800
1000
DOSE (KRAD)
— DRY RESIDUE ZEO-KARB H EXTRACT
---- DRY RESIDUE WATER EXTRACT

Figure 10. Effect of gamma radiation on the jelly grade of pectins extracted from Valencia orange wet residue extracted with Zeo-Karb H and with water.

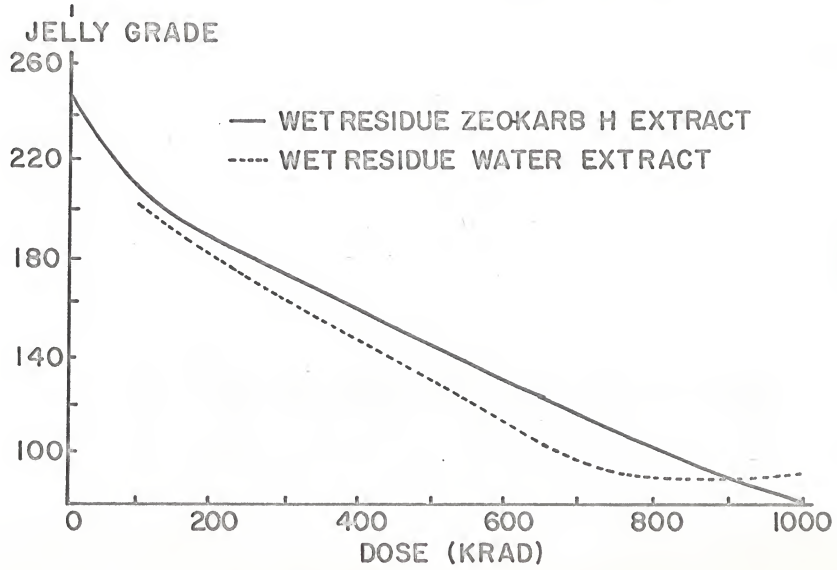

JELLY GRADE
260
220
180
140
100
0
200
400
600
800
1000
DOSE (KRAD)
WET RESIDUE ZEOKARB H EXTRACT
WET RESIDUE WATER EXTRACT

Table 3. Effect of gamma radiation on the jelly grade of pectins extracted from Valencia orange residue.

Analysis of Variance

Source	d.f.	M.S.	F. ratio
Replication	2	1.43	0.08
Residue	3	12376.00	666.85 **
Rep x Res	6	18.56	
Dose	9	15848.99	1102.08 **
Dose x Res	27	520.47	36.19 **
Error	72	14.38	
Total	119		

* Significant at 0.05 level
** Significant at 0.01 level

Table 4. Effect of gamma radiation on the jelly grade of pectins extracted from Valencia orange residue.

Tukey's Test

Dry Residue Water Extraction										
Dose	100	200	300	400	500	600	700	800	900	1000
Mean Jelly Grade	198.0	189.7	186.7	184.0	175.3	171.7	156.3	150.7	120.0	116.7
Statistical Significance at 0.01 level										
Dry Residue Zeo-Karb H Extraction										
Dose	100	300	200	400	600	500	700	800	900	1000
Mean Jelly Grade	212.3	207.3	203.0	201.0	194.7	185.3	173.3	153.3	120.0	117.3
Statistical Significance at 0.01 level										
Wet Residue Water Extraction										
Dose	100	200	300	400	500	600	800	900	1000	700
Mean Jelly Grade	200.0	188.0	169.3	169.0	133.7	112.7	93.3	91.0	91.0	87.0
Statistical Significance at 0.01 level										
Wet Residue Zeo-Karb H Extraction										
Dose	100	200	300	400	500	600	700	800	900	1000
Mean Jelly Grade	203.7	191.7	180.7	177.7	147.0	146.0	116.7	104.0	90.0	85.3
Statistical Significance at 0.01 level										

krad intervals.

Jelly Units

This value was calculated by multiplying yield of pectin by jelly grade. It represents the quantity of jelly that may be produced from a given weight of residue. It is an optimization measure used by the processor.

Jelly units were always higher for the non-irradiated samples. Jelly units were higher in the dry and wet residue Zeo-Karb H extracted than the water extracted samples. This was because of the higher yields at low doses. The wet residue Zeo-Karb H extracted sample had the highest yield and accordingly the highest jelly units at 100 krad (Fig. 11). At 500 krad the dry residue had higher jelly units than the wet residue because the decrease in jelly grade was of lower magnitude than the wet residue. The jelly units of both residues dropped with increased dose because the drop in jelly grade was of higher amplitude than the increase in yield.

In the case of the water extracted residues (Fig. 12) the jelly units were low at the 100 krad irradiation dose due to a low yield. The curves remain almost horizontal with an increase in dose due to a simultaneous decrease in jelly grade and an increase in yield. There was a slight optimum at 500 krad, this optimum was under 30 jelly units, which was too low to consider the residue as a commercial source for pectin. At least 60 jelly units are considered necessary for the commercial production of pectin to be profitable.

Figure 11. Effect of gamma radiation on the jelly units of pectins extracted from Valencia orange dry and wet residue extracted with Zeo-Karb H.

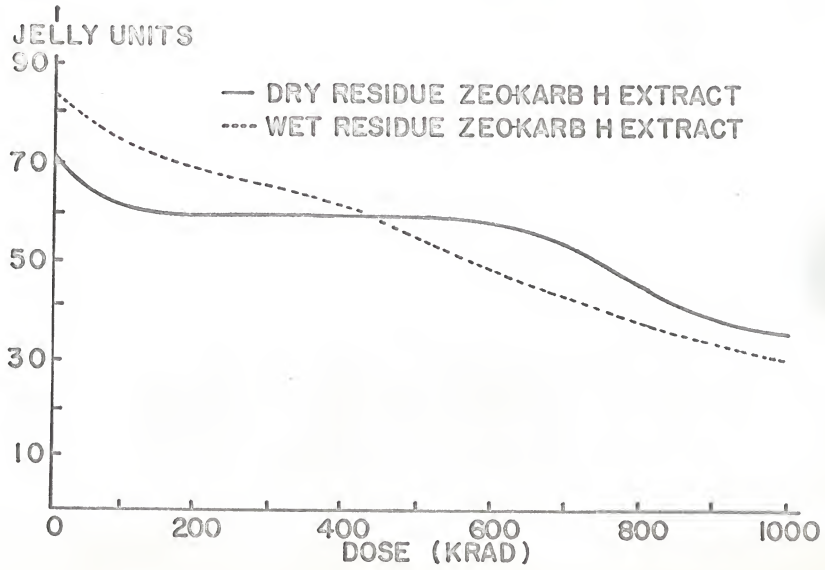
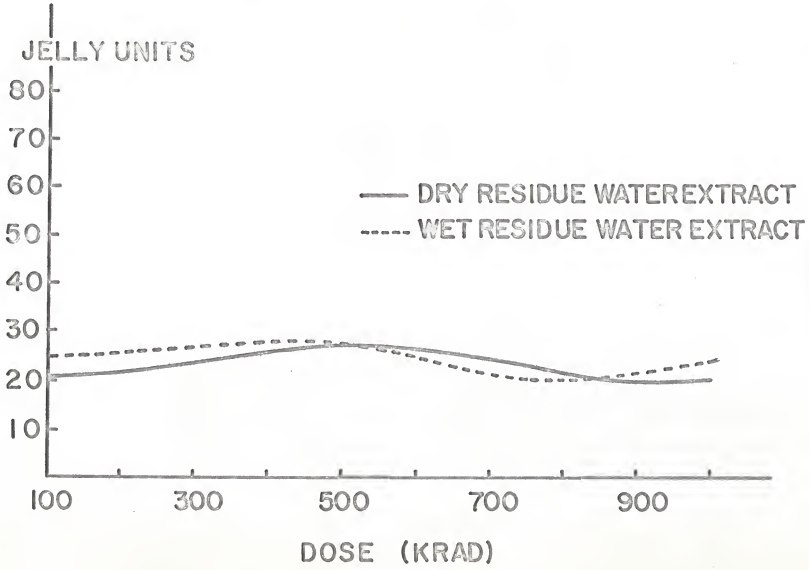

JELLY UNITS
90
70
50
30
10
— DRY RESIDUE ZEOKARB H EXTRACT
---- WET RESIDUE ZEOKARB H EXTRACT
0
200
400
600
800
1000
DOSE (KRAD)

Figure 12. Effect of gamma radiation on the jelly units of pectins extracted from Valencia orange dry and wet residue extracted with water.


JELLY UNITS
80
70
60
50
40
30
20
10
DRY RESIDUE WATER EXTRACT
WET RESIDUE WATER EXTRACT
100
300
500
700
900
DOSE (KRAD)

Figures 13 and 14 show the difference between the water and Zeo-Karb H extracted samples. In the case of the wet residues the Zeo-Karb H extracted sample had a decrease in jelly units, due to the decrease in jelly grade. The same was observed in case of the dry residue water and Zeo-Karb H extracted.

Table 5 shows the analysis of variance and Table 6 Tukey's test. There was a significant difference at the 0.01 level between both doses and extraction of residues and also between their interaction.

Degree of Methylation and Equivalent Weight

Methoxyl content was determined on alcohol precipitates (Fig. 15) and calculated on anhydrogalacturonic acid basis (Fig. 16 and 17) to correct for impurities that are present in the alcohol precipitate. The methoxyl content dropped drastically, then remained constant at about 10% with doses of 400 to 1,000 krad. These results agree with those of Rouse et al. (1968). Kertesz et al. (1956) irradiated pectins and pectin solution but found no significant change in methyl ester content up to 233.7 reps.

Deshpande (1965) stated that the reason for the methoxyl content not to have dropped any further might have been the aggregation of the molecules through linking of the carboxyl groups freed by deesterification. Irradiation could be considered as a method of partial deesterification besides the classical methods in which acids, alkalis or enzymes are used, but it should be remembered that at high doses the molecule is depolymerized. There was little difference between the curves of dry residues or

Figure 13. Effect of gamma radiation on the jelly units of pectins extracted from Valencia orange wet residue extracted with Zeo-Karb H and with water.

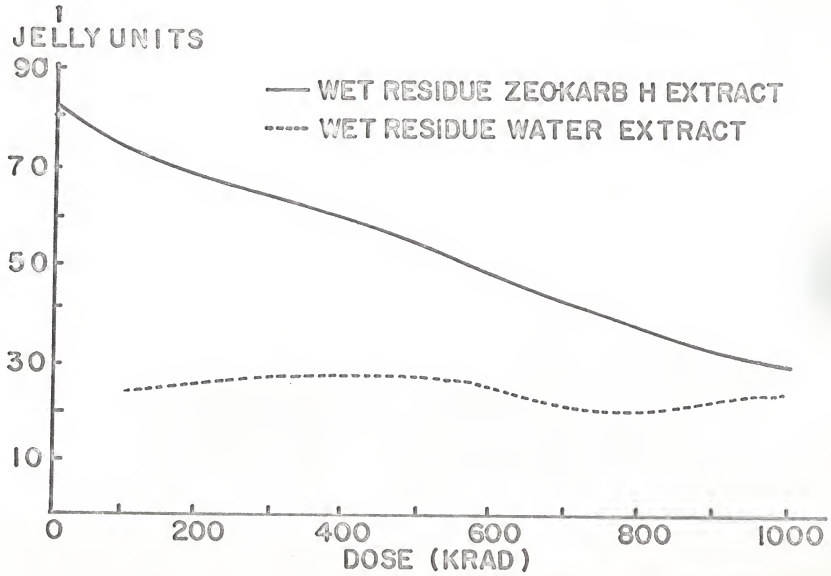

JELLY UNITS
90
70
50
30
10
WET RESIDUE ZEOKARB H EXTRACT
WET RESIDUE WATER EXTRACT
0
200
400
600
800
1000
DOSE (KRAD)

Figure 14. Effect of gamma radiation on the jelly units of pectins extracted from Valencia orange dry residue extracted with Zeo-Karb H and with water.

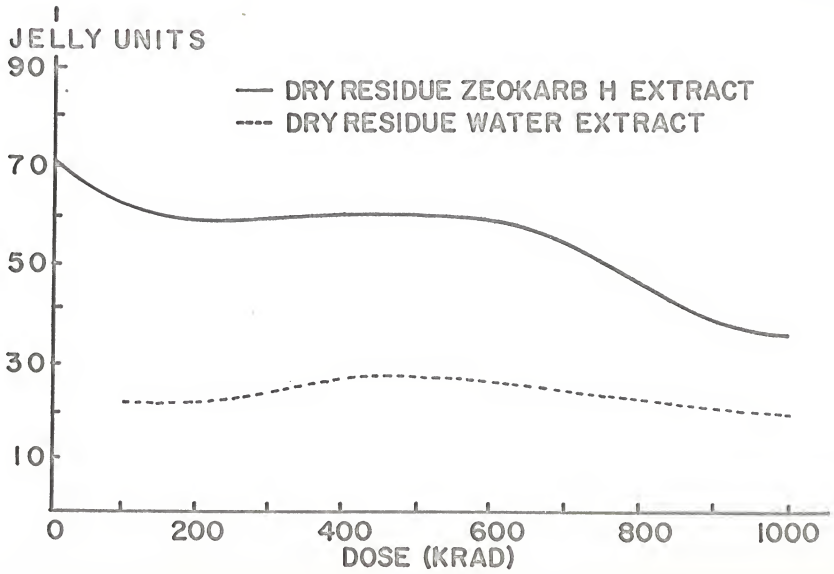

JELLY UNITS
90
70
50
30
10
DRY RESIDUE ZEOKARB H EXTRACT
DRY RESIDUE WATER EXTRACT
0
200
400
600
800
1000
DOSE (KRAD)

Table 5. Effect of gamma radiation on the jelly units of pectins extracted from Valencia orange residue.

Analysis of Variance

Source	d.f.	M.S.	F. ratio
Replication	2	8.93	1.09
Residue	3	9205.73	1005.50 **
Rep x Res	6	8.17	
Dose	9	503.20	146.66 **
Dose x Res	27	159.00	46.34 **
Error	72	3.43	
Total	119		

* Significant at 0.05 level
** Significant at 0.01 level

Table 6. Effect of gamma radiation on the jelly units of pectins extracted from Valencia orange residue.

Tukey's Test

Dry Residue Water Extraction										
Dose	200	900	1000	100	300	800	700	600	500	400
Mean Jelly Units	20.17	20.56	20.79	22.24	23.65	24.40	24.55	25.95	26.43	26.48
Statistical Significance at 0.01 level										
Dry Residue Zeo-Karb H Extraction										
Dose	1000	900	800	700	500	200	400	600	300	100
Mean Jelly Units	36.43	36.91	47.65	55.25	57.11	58.89	60.00	60.03	60.42	62.69
Statistical Significance at 0.01 level										
Wet Residue Water Extraction										
Dose	700	800	1000	900	600	100	300	200	500	400
Mean Jelly Units	20.35	22.47	24.05	24.09	24.70	25.80	26.29	26.63	27.49	27.70
Statistical Significance at 0.01 level										
Wet Residue Zeo-Karb H Extraction										
Dose	1000	900	800	700	600	500	400	300	200	100
Mean Jelly Units	31.87	34.84	39.23	43.41	50.07	53.05	62.18	63.67	69.45	74.33
Statistical Significance at 0.01 level										

Figure 15. Effect of gamma radiation on the methoxyl content of alcohol precipitate extracted from Valencia orange wet and dry residue extracted with Zeo-Karb H and with water.

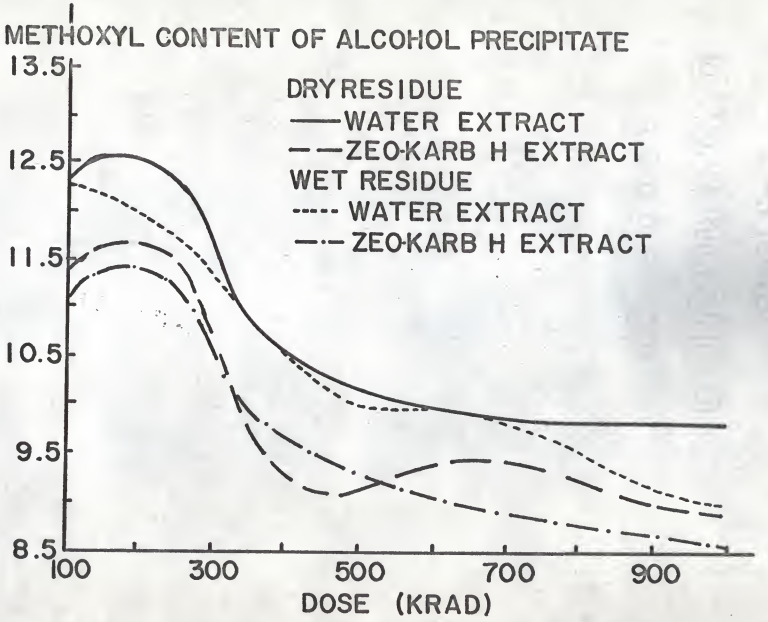

METHOXYL CONTENT OF ALCOHOL PRECIPITATE
13.5
12.5
11.5
10.5
9.5
8.5
DRY RESIDUE
WATER EXTRACT
ZEO-KARB H EXTRACT
WET RESIDUE
WATER EXTRACT
ZEO-KARB H EXTRACT
100
300
500
700
900
DOSE (KRAD)

Figure 16. Effect of gamma radiation on the methoxyl content on AGA basis of pectins extracted from Valencia orange dry and wet residue extracted with Zeo-Karb H.

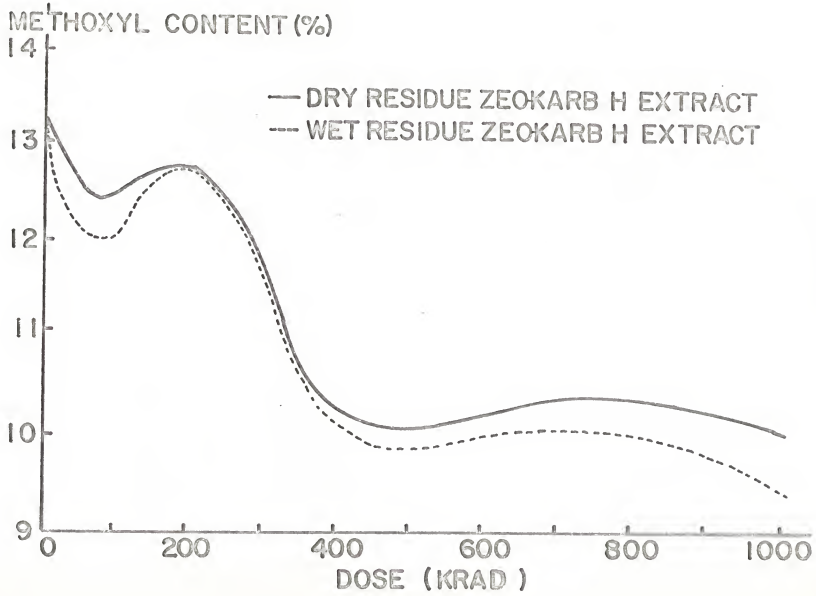
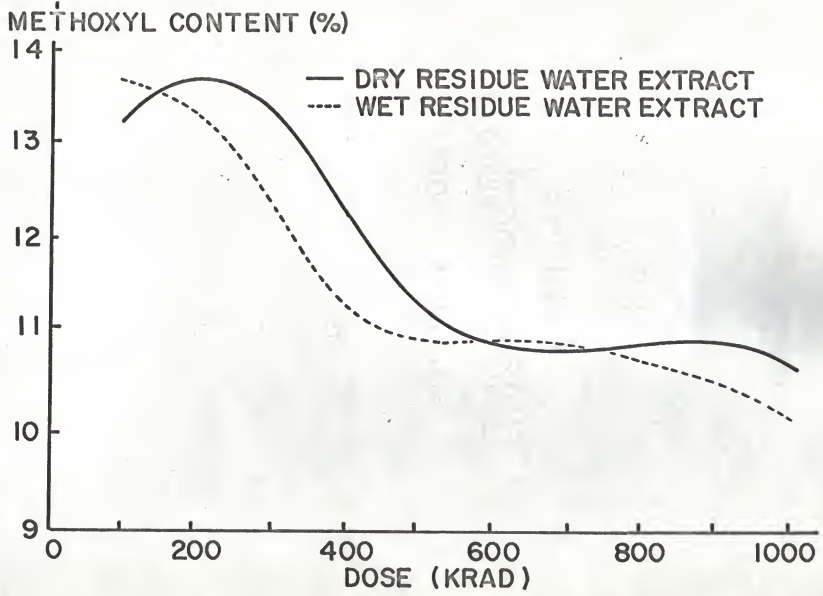

METHOXYL CONTENT (%)
14
13
12
11
10
9
— DRY RESIDUE ZEOKARB H EXTRACT
--- WET RESIDUE ZEOKARB H EXTRACT
0
200
400
600
800
1000
DOSE (KRAD)

Figure 17. Effect of gamma radiation on the methoxyl content on AGA basis of pectins extracted from Valencia orange dry and wet residue extracted with water.


METHOXYL CONTENT (%)
14
13
12
11
10
9
0
200
400
600
800
1000
DOSE (KRAD)
DRY RESIDUE WATER EXTRACT
WET RESIDUE WATER EXTRACT

wet residues, Zeo-Karb H (Fig. 16) or water (Fig. 17) extracted samples. Methoxyl content was consistently lower in the case of the Zeo-Karb H extracted samples, whether extracted from wet (Fig. 18) or dry (Fig. 19) residues. Gaddum (1934) noticed that water extracted pectin from lemon albedo had a higher methoxyl content than that extracted with acid. From the statistical analysis shown in Table 7 there was a significant difference at the 0.01 level between both doses and extraction of residues, while their interaction was nonsignificant. This means that irradiation affects methoxyl content to a similar amplitude for all extraction methods examined. From Tukey's test (Table 8) it can be seen that there were no significant differences between methoxyl content of all samples treated between 100 and 200 krad.

Data on equivalent weight were not reported because it can be reliable only if certain corrections were introduced (Olsen et al., 1939).

Purity of Pectin as Anhydrogalacturonic Acid

The data on the percentage anhydrogalacturonic acid of pectins extracted were not presented because there were no significant differences between all samples. This probably indicates that even though the polymer is degraded, the monomer galacturonide is not altered. Purity ranged between 88 and 92%.

Relative Viscosity

Relative viscosities of 0.5% solutions were measured, these reflect jelly grade or average molecular weight. Relative viscosities

Figure 18. Effect of gamma radiation on the methoxyl content on AGA basis of pectins extracted from Valencia orange wet residue extracted with Zeo-Karb H and with water.

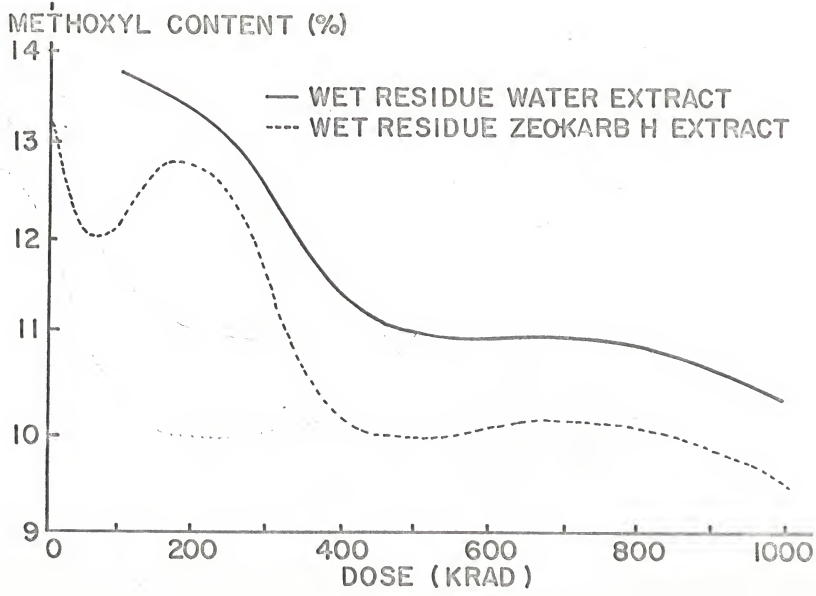

METHOXYL CONTENT (%)
14
13
12
11
10
9
0
200
400
600
800
1000
DOSE (KRAD)
— WET RESIDUE WATER EXTRACT
---- WET RESIDUE ZEOKARB H EXTRACT

Figure 19. Effect of gamma radiation on the methoxyl content on AGA basis of pectins extracted from Valencia orange dry residue extracted with Zeo-Karb H and with water.

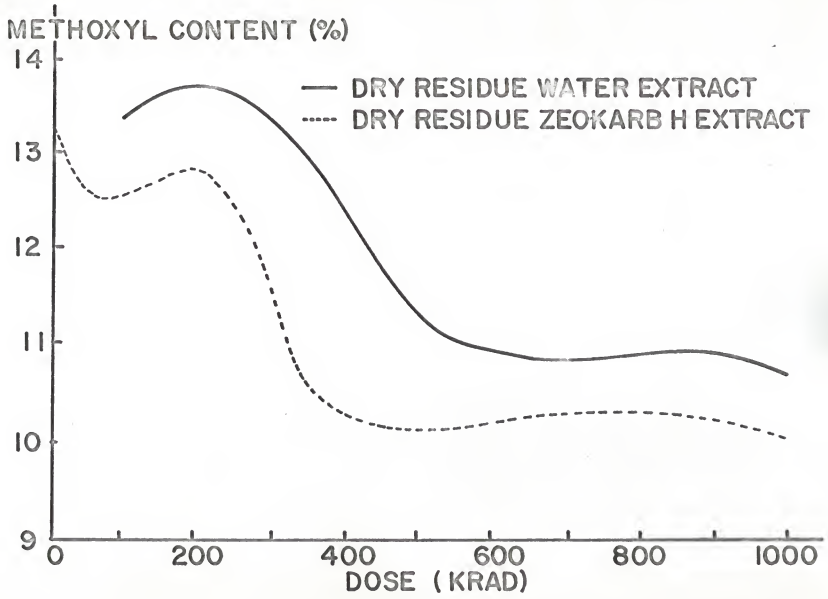

METHOXYL CONTENT (%)
14
13
12
11
10
9
0
200
400
600
800
1000
DOSE (KRAD)
DRY RESIDUE WATER EXTRACT
DRY RESIDUE ZEOKARB H EXTRACT

Table 7. Effect of gamma radiation on the methoxyl content on AGA basis of pectins extracted from Valencia orange residue.

Analysis of Variance

Source	d.f.	M.S.	F. ratio
Replication	2	0.30	2.21
Residue	3	7.53	55.79 **
Rep x Res	6	0.14	
Dose	9	15.09	60.11 **
Dose x Res	27	0.27	1.06
Error	72	0.25	
Total	119		

* Significant at 0.05 level
** Significant at 0.01 level

Table 8. Effect of gamma radiation on the methoxyl content on AGA basis of pectins extracted from Valencia orange residue.

	Tukey's Test									
Dry Residue Water Extraction										
Dose	300	800	1000	700	500	900	600	400	100	200
Mean Methoxyl (%)	10.56	10.57	10.77	10.85	10.96	11.03	11.28	12.10	13.15	13.91
Statistical Significance at 0.01 level										
Dry Residue Zeo-Karb H Extraction										
Dose	300	900	500	1000	400	800	600	700	100	200
Mean Methoxyl (%)	10.07	10.10	10.13	10.19	10.24	10.27	10.34	10.58	12.18	12.96
Statistical Significance at 0.01 level										
Wet Residue Water Extraction										
Dose	900	1000	300	700	500	800	600	400	200	100
Mean Methoxyl (%)	9.46	10.26	10.28	10.75	10.93	10.95	11.07	11.29	13.47	13.71
Statistical Significance at 0.01 level										
Wet Residue Zeo-Karb H Extraction										
Dose	1000	500	900	300	800	700	600	400	100	200
Mean Methoxyl (%)	9.65	9.69	9.78	9.79	9.93	10.07	10.15	10.29	11.98	12.96
Statistical Significance at 0.01 level										

were also used to assume approximate jelly grade.

Relative viscosities decreased in all samples with increase in dose. The relative viscosity of pectin solutions extracted from dry residue, each with the Zeo-Karb H and with water, decreased 59.9% and 55.9%, respectively, when irradiated from 100 to 1,000 krad (Fig. 20). A similar situation was found for the pectins extracted from wet residues. This decrease was 73.1% in the Zeo-Karb H extracted sample and 66.5% in the water extracted material (Fig. 21). A greater percent reduction in viscosity of the pectin solutions was observed when Zeo-Karb H was used as the extractant, because it will remove cations, such as calcium and magnesium, which the water does not. These cations contribute to the relative viscosity of the solution.

In comparing the relative viscosities of pectin solutions extracted with both Zeo-Karb H (Fig. 22) and with water (Fig. 23) from dry and wet residues, it was found that the pectin from wet residues had a lower relative viscosity than those of the dry sample. This showed once again that irradiation will affect a wet material more than it does a dry one.

As seen from Table 9 there was a significant difference at the 0.01 level between doses and between extraction of residues, also between the interaction. From Tukey's test (Table 10) it can be seen that the significant difference is at 100 to 200 krad intervals.

Figure 20. Effect of gamma radiation on the relative viscosity of pectins extracted from Valencia orange dry residue extracted with Zeo-Karb H and with water.

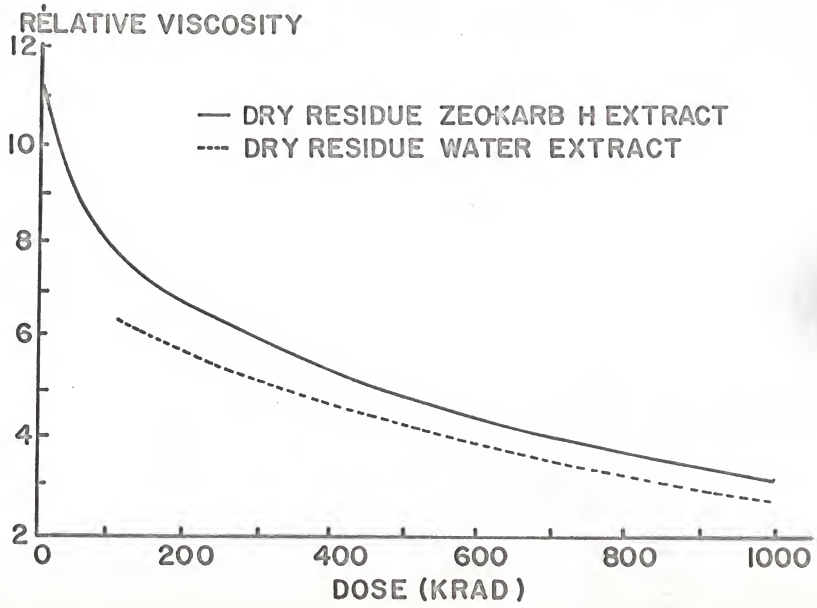

RELATIVE VISCOSITY
12
10
8
6
4
2
— DRY RESIDUE ZEOKARB H EXTRACT
---- DRY RESIDUE WATER EXTRACT
0
200
400
600
800
1000
DOSE (KRAD)

Figure 21. Effect of gamma radiation on the relative viscosity of pectins extracted from Valencia orange wet residue extracted with Zeo-Karb H and with water.

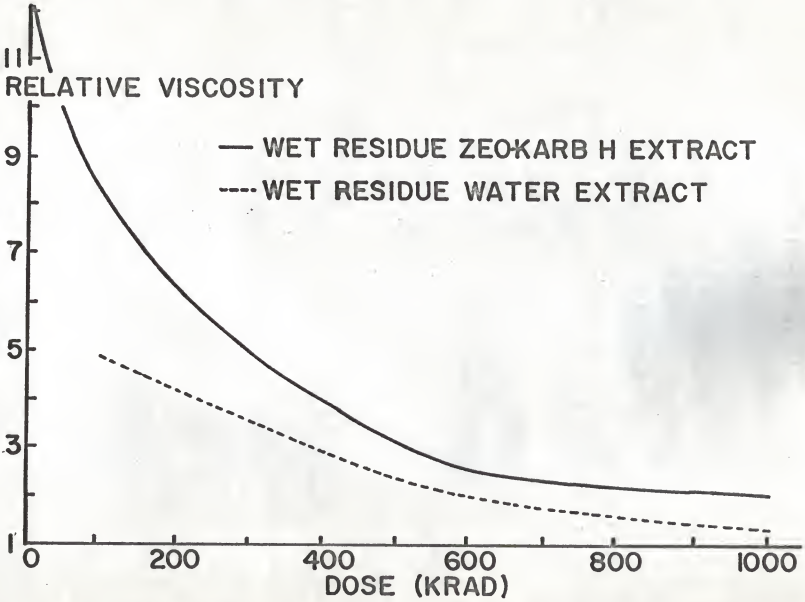

RELATIVE VISCOSITY
11
9
7
5
3
1
— WET RESIDUE ZEOKARB H EXTRACT
---- WET RESIDUE WATER EXTRACT
0
200
400
600
800
1000
DOSE (KRAD)

Figure 22. Effect of gamma radiation on the relative viscosity of pectins extracted from Valencia orange dry and wet residue extracted with Zeo-Karb H.

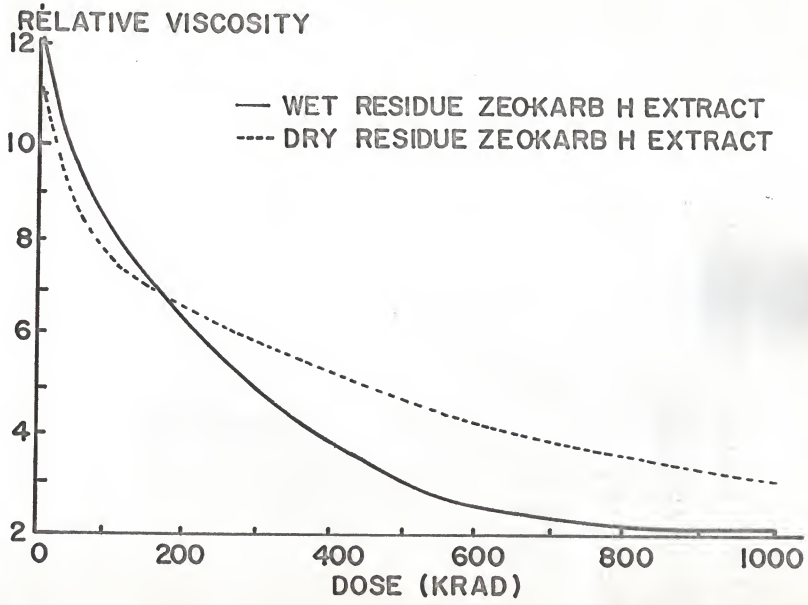

RELATIVE VISCOSITY
12
10
8
6
4
2
0
200
400
600
800
1000
DOSE (KRAD)
— WET RESIDUE ZEOKARB H EXTRACT
---- DRY RESIDUE ZEOKARB H EXTRACT

Figure 23. Effect of gamma radiation on the relative viscosity of pectins extracted from Valencia orange dry and wet residue extracted with water.

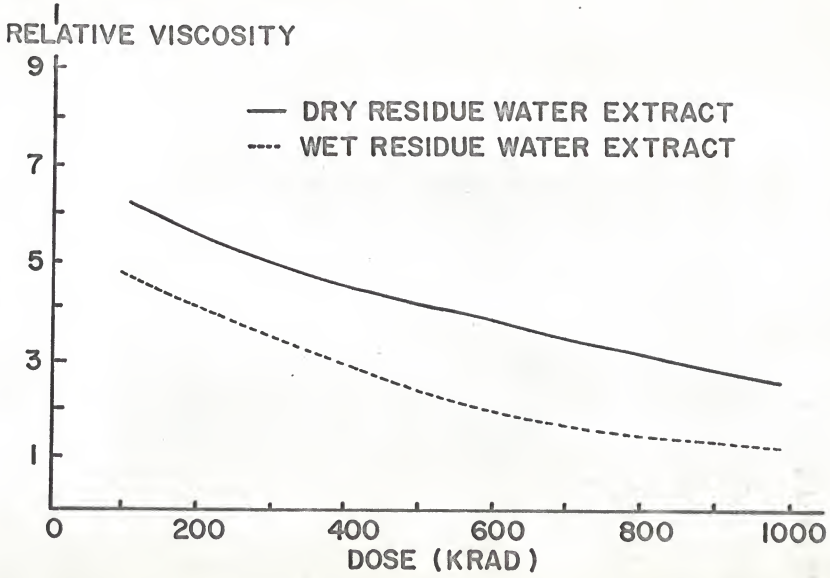

RELATIVE VISCOSITY
9
7
5
3
1
DRY RESIDUE WATER EXTRACT
WET RESIDUE WATER EXTRACT
0
200
400
600
800
1000
DOSE (KRAD)

Table 9. Effect of gamma radiation on the relative viscosity of pectins extracted from Valencia orange residue.

Analysis of Variance			
Source	d.f.	M.S.	F. ratio
Replication	2	0.95	6.30 *
Residue	3	29.51	196.42 **
Rep x Res	6	0.15	
Dose	9	24.53	179.37 **
Dose x Res	27	10.86	7.94 **
Error	72	0.14	
Total	119		

* Significant at 0.05 level
** Significant at 0.01 level

Table 10. Effect of gamma radiation on the relative viscosity of pectins extracted from Valencia orange residue.

Tukey's Test

Dry Residue Water Extraction										
Dose	1000	900	800	700	600	500	300	400	200	100
Mean Rel. Visc.	2.62	2.80	3.47	3.73	4.27	4.44	5.11	5.19	5.20	5.96
Statistical Significance at 0.01 level										
Dry Residue Zeo-Karb H Extraction										
Dose	1000	900	800	700	600	500	400	300	200	100
Mean Rel. Visc.	3.07	3.33	3.73	4.13	4.36	4.98	5.33	6.00	6.76	7.64
Statistical Significance at 0.01 level										
Wet Residue Water Extraction										
Dose	900	1000	700	800	600	500	400	300	200	100
Mean Rel. Visc.	1.38	1.42	1.73	1.82	2.04	2.44	2.98	3.60	4.13	4.26
Statistical Significance at 0.01 level										
Wet Residue Zeo-Karb H Extraction										
Dose	900	800	1000	700	600	500	400	300	200	100
Mean Rel. Visc.	2.04	2.26	2.27	2.44	2.58	3.02	4.09	5.16	6.31	8.40
Statistical Significance at 0.01 level										

SUMMARY AND CONCLUSIONS

Investigations were initiated to determine the possibility of using ionizing radiation for the hydrolysis of the pectic constituents of wet and dry residues from Valencia oranges, and the effect of the treatment on the characteristics of the extracted pectins. The irradiation doses were from 0 to 1,000 krad with 100 krad increments, and the quality factors of primary interest were percentage yield, jelly grade, jelly units, methoxyl content and relative viscosity.

Irradiated wet residues had a higher yield, but a lower grade than the dry.

1. Percentage yield increased with irradiation dose, but did not reach the level of the non-irradiated Zeo-Karb H extracted samples. Therefore, it was concluded that irradiation hydrolyzes the protopectin in the residue and could be used for this purpose.

2. Jelly grade decreased with increasing dose and it was postulated that this is due to a depolymerization of the pectinic acid molecule.

3. Jelly units were lower in the irradiated samples than in the non-irradiated; and at all treatments the jelly units were under 30. This makes the use of ionizing radiation as a means

of hydrolysis for the commercial production of pectins from Valencia orange residue non-profitable.

4. Methoxyl content decreased with increasing dose, but remained constant at about 10% with doses of 400 to 1,000 krad. Irradiation could be considered as a method of partial deesterification for the processing of an inexpensive pectin (not including the cost of the irradiation unit) to be used as a thickening agent for beverages and creams.

5. Relative viscosity of the pectin solutions decreased with increasing dose, which led to the conclusion that the main effect of irradiation on pectinic acid molecules is depolymerization.

APPENDIX : DETAILED DATA OF FIGURES 3 to 23.

Table 11. Effect of gamma radiation on the yield of pectins extracted from Valencia orange residue.

	Method of Extraction			
	Dry Residue		Wet Residue	
Dose (krad)	Water Extract (%)	Zeo-Karb H Extract (%)	Water Extract (%)	Zeo-Karb H Extract (%)
0	----	29.00	----	34.72
100	11.21	29.52	12.88	36.49
200	10.63	28.99	14.16	36.24
300	12.66	29.13	15.52	35.01
400	14.40	29.85	18.59	35.00
500	15.09	30.80	20.57	36.08
600	15.12	30.83	21.90	34.31
700	15.70	31.88	23.47	37.19
800	16.19	31.07	24.18	37.72
900	17.63	30.76	26.63	38.71
1000	18.50	31.05	26.47	37.33

Table 12. Effect of gamma radiation on the jelly grade of pectins extracted from Valencia orange residue.

	Method of Extraction			
	Dry Residue		Wet Residue	
Dose (krad)	Water Extract	Zeo-Karb H Extract	Water Extract	Zeo-Karb H Extract
0	----	245.00	----	246.00
100	198.00	212.33	200.00	203.66
200	189.66	203.00	188.00	191.66
300	186.66	207.33	169.33	180.66
400	184.00	201.00	149.00	177.66
500	175.33	185.33	133.66	147.00
600	171.66	194.66	112.66	146.00
700	156.33	173.33	87.00	116.66
800	150.66	153.33	93.33	104.00
900	120.00	120.00	91.00	90.00
1000	116.66	117.33	91.00	85.33

Table 13. Effect of gamma radiation on the jelly units of pectins extracted from Valencia orange residue.

	Method of Extraction			
	Dry Residue		Wet Residue	
Dose (krad)	Water Extract	Zeo-Karb H Extract	Water Extract	Zeo-Karb H Extract
0	----	71.00	----	85.40
100	22.23	62.69	25.79	74.33
200	20.16	58.89	26.62	69.45
300	23.65	60.41	26.29	63.27
400	26.48	60.00	27.69	62.18
500	26.42	57.11	27.49	53.04
600	25.94	60.03	24.69	50.07
700	24.54	55.25	20.34	43.40
800	24.40	47.64	22.47	39.22
900	20.56	36.91	24.18	34.84
1000	20.79	36.43	24.04	31.86

Table 14. Effect of gamma radiation on the methoxyl content of alcohol precipitate from Valencia orange residue.

	Method of Extraction			
	Dry Residue		Wet Residue	
Dose (krad)	Water Extract (%)	Zeo-Karb H Extract (%)	Water Extract (%)	Zeo-Karb H Extract (%)
0	----	12.28	----	11.97
100	12.00	11.17	12.25	10.94
200	12.70	11.83	12.12	11.58
300	9.37	9.06	9.39	8.75
400	10.77	9.07	9.81	9.18
500	9.70	9.05	9.91	8.65
600	10.00	9.38	9.96	9.14
700	9.84	9.59	9.68	8.99
800	9.30	8.90	9.83	8.80
900	9.86	9.08	8.96	8.67
1000	9.77	8.89	9.03	8.48

Table 15. Effect of gamma radiation on the methoxyl content on AGA basis of pectins extracted from Valencia orange residue.

	Method of Extraction			
	Dry Residue		Wet Residue	
Dose (krad)	Water Extract (%)	Zeo-Karb H Extract (%)	Water Extract (%)	Zeo-Karb H Extract (%)
0	----	13.28	----	13.30
100	13.14	12.17	13.71	11.98
200	13.91	12.95	13.47	12.96
300	10.56	10.07	10.28	9.79
400	12.10	10.24	11.28	10.29
500	10.96	10.12	10.93	9.68
600	11.28	10.34	11.06	10.15
700	10.85	10.57	10.75	10.06
800	10.57	10.27	10.95	9.92
900	11.03	10.09	9.96	9.78
1000	10.77	10.18	10.26	9.64

Table 16. Effect of gamma radiation on the relative viscosity of pectins extracted from Valencia orange residue.

	Method of Extraction			
	Dry Residue		Wet Residue	
Dose (krad)	Water Extract	Zeo-Karb H Extract	Water Extract	Zeo-Karb H Extract
0	----	11.19	----	12.08
100	5.96	7.64	4.25	6.40
200	5.20	6.75	4.13	6.31
300	5.11	6.00	3.60	5.15
400	5.17	5.33	2.97	4.08
500	4.44	4.97	2.44	3.02
600	4.26	4.35	2.04	2.57
700	3.73	4.13	1.73	2.44
800	3.46	3.73	1.82	2.26
900	2.79	3.33	1.37	2.04
1000	2.62	3.06	1.42	2.26

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BIOGRAPHICAL SKETCH

The author was born March 9, 1939, in Cairo, Egypt. He was graduated from Victoria College, Meadi, Egypt, in 1957. He was enrolled at Cairo University from 1957 until he received his B. Sc. degree in Agriculture in 1962. He then worked for the Tractor and Engineering Co. S. A. E. In 1963, he came to the United States, enrolled at the University of Tennessee, and obtained his M. S. degree in 1965 from the Department of Food Technology. He then entered the University of Florida in 1966, and has been working toward the degree of Doctor of Philosophy.

He is a member of Alpha Zeta, Phi Sigma and Gamma Sigma Delta honorary fraternities. He is also a member of the Institute of Food Technologists.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

March, 1969

Dean, College of Agriculture

Dean, Graduate School

Supervisory Committee:

Chairman